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THE JOURNAL OF HYGIENE

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IS THE NEURITIS-PREVENTING VITAMINE CONCERNED IN CARBOHYDRATE METABOLISM?

BY EDWARD B. VEDDER,

Lt-Colonel, Medical Corps, U.S. Army.

VEDDER and CLARK (1912), reporting observations on polyneuritis gallinarum based on experiments lasting for several years during which time over 200 fowls were used, make the following statement with regard to the relation between the amount of polished rice eaten and the time of development of neuritis.

It has been generally observed that the great majority of fowls fed on polished rice usually lose their appetites after about a week on this diet, and thereafter eat only small amounts of rice. There are always a few fowls however which eat greedily up to the very last, and will eat far greater amounts than the usual ration allowed. Several deductions have been drawn from this fact with regard to the development of neuritis. Some observers have thought that those fowls that have eaten well throughout the experiment have been protected from the development of the disease by this increased consumption of rice, and therefore have been inclined to regard polyneuritis as the result of simple inanition. On the other hand, other observers have thought that those fowls that ate the most rice developed the disease soonest, and have regarded this as an argument in favour of the theory that polyneuritis gallinarum is caused by some toxin contained in the polished rice. We have observed fowls that always ate well, and yet developed neuritis sooner than usual, and we have observed fowls that ate large quantities of rice throughout the experiment, but whose depletion period was longer than normal. Again some of the fowls that have eaten poorly have developed neuritis promptly, while others have not developed the disease at all. Therefore it is believed that the amount of rice eaten has little to do with the development of the disease, which depends rather on the idiosyncrasy of the fowl with regard to the amount of neuritis-preventing substance required.

Later, Braddon and Cooper (1914) and Funk (1914) published experiments which they thought demonstrated that when fowls or pigeons were fed on polished rice or starch, with or without a fixed amount of anti-neuritic vitamine, the greater the proportion of carbohydrate in the food, the quicker was the onset of polyneuritis. From this evidence these investigators concluded that the vitamine played an active part in the metabolism of carbohydrates.

Here is a direct contradiction as to the experimental facts, for the statement of Vedder and Clark cannot be reconciled with the statements and experiments of Braddon and Cooper and of Funk. The subject has been deemed worthy of further investigation for it is a matter of considerable scientific and practical importance. It is obviously important for the physiologist to determine the exact rôle of the vitamines in metabolism. The sanitarian must also have exact information as to the amount of vitamine required in order that he may prescribe a diet that will always prevent beriberi. The writer has suggested in various publications that the antineuritic vitamine acts as a building stone which is essential for the metabolism of certain tissues of the body and more particularly of the nervous system. If this view is correct, it follows that each individual requires a definite amount of vitamine irrespective of the amount of carbohydrate eaten. The amount required varies somewhat for individuals depending upon personal idiosyncrasy, the amount of labour performed and other circumstances, but this variation is relatively slight. That is in practical terms, if a certain definite amount of beans or rice polish supplying the vitamine be consumed, and this amount be sufficient to supply the demand for vitamine, beriberi will never develop no matter how large the amount of rice or other carbohydrate consumed in the ration.

On the other hand if the view advocated by Braddon and Cooper and by Funk is correct, it follows that the more carbohydrate is eaten, the more vitamine must be supplied; thus if four ounces of beans are prescribed as a preventive, this might be sufficient to prevent beriberi while one pound of polished rice is eaten daily, but if two pounds of rice were eaten daily with the same quantity of beans, the individual would be in danger of developing beriberi.

I have endeavoured to perform some experiments that would throw more light on this matter, and explain the apparent conflict in the experimental facts.

Experiment 1. This experiment was performed in collaboration with Mr Rommel, Chief of the division of Animal Husbandry of the U.S. Department of Agriculture. The experiment was outlined under the direction of Mr Rommel and myself, but the observations were made and recorded by unprejudiced workers who did not know what the experiment was intended to prove. On January 18, 1916, six young cockerels were placed in individual coops and fed on an exclusive diet of polished rice. The birds were allowed to eat all they desired, but the amount supplied each day was weighed into special feed boxes so constructed

that no rice could be lost, and the amount remaining uneaten was weighed at the end of each day, so that the actual amount consumed by each bird was recorded every day. The fowls were weighed at the beginning and at the end of the experiment, and the time at which symptoms of paralysis appeared were carefully noted. On February 22, 1916, the experiment was repeated using five cocks. The results of this experiment are shown in Table I, in which all the birds used are arranged in order

TABLE I.

Fowl no.	Original weight	Total amount of rice eaten before development of neuritis	Percent. of total rice eaten before development of neuritis compared to original body weight	Average daily amount of rice eaten	Percent. of body weight eaten daily	No. of days after experiment began when neuritis developed	Weight lost	Remarks
1	3600	2647	73.5	132.3	3.67	20	96	Moderate paralysis
2	1824	1095	60.0	64.4	3.53	17	312	Marked paralysis
3	3216	2271	70.6	98.7	3.06	23	192	Symptoms so slight that they could only be detected on careful examination
4	3744	2535	67.7	105.6	2.82	24	95	Symptoms slight
5	2640	1665	63.0	69.3	2.62	24	297	Paralysis pronounced. Died of polyneuritis three days later
6	3104	1355	43.6	67.7	2.18	20	965	Paralysis marked but not complete. Bird could move when stirred
7	3456	1611	46.6	70.0	2.02	23	750	Symptoms trifling. Could stand and walk but with evidence of paralysis
8	2832	1113	39.3	53.0	1.87	21	961	Symptoms only slight paralysis of legs
9	3840	1275	33.2	70.8	1.84	18	960	Rapid and complete paralysis
10	3744	1171	31.2	53.2	1.42	22	744	Symptoms of neuritis mild
11	2304	690	29.9	32.8	1.42	21	480	Moderate paralysis

depending upon the percentage of rice eaten as compared with their original body weight. From this table it is possible to see at a glance the lack of relation between the amount of rice eaten and the time of development of neuritis. All figures for the weights of the fowls and amounts of rice eaten are expressed in grams.

This experiment indicates that when fowls are fed on polished rice *ad libitum*, the rapidity of onset of polyneuritis bears no relation to the amount of rice eaten. Certainly it is evident that neuritis does not develop more quickly in those birds that eat the largest amounts of rice. This bears out my previous observations, which were also made on birds allowed to eat as much rice as they desired.

Funk and Braddon and Cooper on the other hand fed their birds by hand. Thus Funk (1914) performed the following experiment. Doves

were fed by hand on polished rice in various quantities. One group received one half a gram daily, another group 5 grams daily and still other groups 10 and 20 grams daily. Funk stated that the doves receiving one half a gram of rice died of simple starvation without a trace of polyneuritis, while the result of the experiment on the remainder of the doves was as follows:

	5 grams	10 grams	20 grams
Appearance of polyneuritis	39 days	36 days	22 days
Death	42 „	38 „	22 „

I repeated this experiment but used fowls because they are easier to feed by hand.

Experiment 2. Twelve fowls of the same breed and approximately the same size and age were divided into three groups. An endeavour was made to feed the first group 100 grams of rice daily, the second group 50 grams of rice daily, and the third group 25 grams of rice daily. Polished rice was used and the birds were hand fed. However it was very soon found that it was impracticable to feed the fowls in Group 1 as much as 100 grams of rice daily. After a few days of such feeding they were unable to digest this amount of rice with sufficient rapidity, and the rice accumulated in their crops. If feeding was then continued, after a few days more their crops became so distended with rice that the introduction of more rice became impossible without killing the bird by the pressure developed by the swelling rice. As soon as it was found that the proposed amounts of rice could not be fed without mechanically killing the birds, the amount actually administered was weighed, and the actual amounts of rice so fed are indicated in Table II in comparison with the amounts originally proposed.

TABLE II.

No. of fowl	Proposed feeding		Amount actually fed					Result of experiment
	Original weight	Daily amount (grams)	Percent. of body weight to be fed daily	Total amount polished rice fed prior to death	Percent. of body weight fed before death	Average daily amount of rice fed (grams)	Percent. body weight fed daily	
1	942	100	10.61	1200	127.3	85.7	9.09	Died 14 days after exp. started
2	1290	100	7.75	1250	96.9	73.5	5.69	„ 17 „ „
3	1292	100	7.75	1325	102.5	69.7	5.39	„ 19 „ „
4	1595	100	6.26	1400	87.7	73.6	4.60	„ 19 „ „
5	1100	50	4.5	850	77.2	44.7	4.06	„ 19 „ „
6	1075	50	4.6	900	83.7	45.0	4.01	„ 20 „ „
7	1315	50	3.8	1150	87.2	50.0	3.80	„ 23 „ „
8	1505	50	3.3	800	53.1	42.1	2.79	„ 19 „ „
9	1132	25	2.2	600	53.7	25.0	2.20	Polyneuritis after 24 days
10	1249	25	2.0	425	34.2	21.2	1.69	„ „ 20 „
11	1244	25	2.0	—	—	—	—	No polyneuritis after 32 days when the experiment was discontinued
12	1292	25	1.9	—	—	—	—	

The results of this experiment agree with those obtained by Funk and by Braddon and Cooper in so far as they refer to the death of the birds. That is, the birds receiving the largest amounts of rice died first. But it was very evident that they did not die of polyneuritis. After about two weeks, the birds receiving the larger amounts of rice lay prostrated in the cages, too weak to stand up, but they did not show any symptoms of paralysis of the legs or retraction of the neck. Their crops were enormously distended. I am satisfied that the first four birds of the series died from the results of over-feeding, and that had the full 100 grams been fed daily, their death would have been brought about even more swiftly. It seems probable that Funk's results are to be explained in the same way. Funk recognized the fact that his doves fed on one-half a gram of rice daily died of starvation rather than of polyneuritis. It is quite as possible to kill an animal by overfeeding as by underfeeding.

The experiments of Braddon and Cooper are open to the same criticism; in some of them the birds were fed as much as one-fifth or 20 per cent. of their body weight daily, while it appears that fowls will not eat more than 4 per cent. of their body weight voluntarily, and that they are killed (without polyneuritis) by forced feeding of polished rice in amounts ranging from 6 to 9 per cent. of their body weight. It should be stated that the nerves of my fowls were examined and a certain degree of degeneration was found by the Marchi method. However in no case was this comparable to the extent of degeneration found in the nerves of fowls which have succumbed to polyneuritis, and it must be remembered that Vedder and Clark showed that degeneration began in the nerves of fowls after only seven days feeding on polished rice. Degeneration begins before the symptoms of polyneuritis appear, and continues after the bird is cured, so that the finding of moderate degrees of degeneration is no proof that the bird died of polyneuritis, and cannot be used to controvert the opinion that the cause of death in these experiments was overfeeding and not polyneuritis.

In order to confirm this conclusion, I repeated the experiment using unpolished rice. Twelve fowls were divided into three groups. The first group was fed 150 grams of unpolished rice daily, the second 75 grams, and the third group 50 grams. Table III shows the amounts fed in proportion to the body weight, and the results of the experiment.

TABLE III.

No. of fowl	Original weight	Daily amount of unpolished rice fed	Percent. of body weight fed daily	Results
1	1580	150	9.49	Residue accumulated in crop after 19 days feeding. Alive at end of 30 days when experiment was discontinued. Would probably have died had feeding been continued
2	1630	150	9.20	do. do. do.
3	1710	150	8.77	Residue accumulated in crop after 11 days feeding. Died after 23 days feeding, with crop greatly distended
4	1810	150	8.28	Residue accumulated in crop after 11 days feeding. Died after 24 days feeding, with crop greatly distended
5	1550	75	4.83	Remained well for 30 days when feeding was discontinued
6	1580	75	4.74	do. do. do.
7	1610	75	4.65	do. do. do.
8	1740	75	4.31	Residue accumulated in crop after 15 days feeding. Died after 29 days feeding, crop greatly distended
9	1220	50	4.09	Remained well for 30 days when feeding was discontinued
10	1530	50	3.26	do. do. do.
11	1560	50	3.20	do. do. do.
12	1690	50	2.95	do. do. do.

Since birds fed on unpolished rice never develop polyneuritis, the death of these birds when fed from 4 to 9 per cent. of their body weight of unpolished rice, indicates that these birds were killed by overfeeding. The results obtained are somewhat irregular and it may be seen that the birds receiving the largest amounts did not in all cases show distress first. This is probably to be explained on the basis of individual idiosyncrasy. It is well known that weight for weight some individuals eat more than others. It is probable also that the results would have been more consistent had the experiment been continued a little longer. However the experiment was so revolting that it was only continued until sufficient information was obtained to prove definitely that birds can be killed by overfeeding on a diet that cannot produce polyneuritis.

Experiment 3. If polyneuritis develops in birds fed on food containing no carbohydrate, such as sterilized meat or sterilized egg, this would appear to show that the antineuritic vitamine is essential to the body in some way other than as a factor in carbohydrate metabolism. Accordingly meat and eggs were sterilized in the autoclave at 120° C. for half-an-hour, since many experiments have indicated that the antineuritic vitamine is destroyed in most foods at this temperature. Four fowls were fed on a daily ration of 50 grams of sterilized egg, half white and half yolk, and another group of fowls was fed on a daily ration of 50 grams of sterilized meat. The results of this experiment are indicated in Table IV.

TABLE IV.

Fowl	Food	Developed neuritis	Remarks
1	egg		Died in 16 days with inflammation of crop
2	"	56 days	Completely paralyzed in 58 days, and died in 63 days
3	"	63 "	" " " 69 " " 71 "
4	"	64 "	Completely paralyzed in 70 days. Treated with autolyzed yeast extract and recovered
1	meat	70 "	Completely paralyzed in 72 days, and died in 75 days
2	"	73 "	" " " 79 " " 83 "
3	"	95 "	" " " 101 " " 102 "
4	"	101 "	" " " 112 " " 114 "

From this it will be seen that fowls fed on sterilized egg or sterilized meat develop polyneuritis with great regularity, but only after a considerably longer depletion period than is observed in fowls fed exclusively on polished rice. This may be because the *v*itamine is not entirely destroyed in these foods by the sterilization, or because nutrition may be maintained better on these foods even in the absence of *v*itamine, than is the case when rice alone is fed. The nerves of all these fowls were examined by the Marchi method and showed the typical degeneration that accompanies polyneuritis gallinarum. Since there was no carbohydrate whatever in the diet of these fowls, we must conclude that the antineuritic *v*itamine is essential to the body in some other manner than as a factor in carbohydrate metabolism.

Experiment 4. If the antineuritic *v*itamine is concerned in carbohydrate metabolism, it might be expected that fowls fed on a considerable amount of polished rice in addition to sterilized meat or egg, would develop polyneuritis faster than birds fed on sterilized meat or egg alone. Accordingly four fowls were fed on a ration of 25 grams of polished rice and 25 grams of sterilized egg. Four other fowls were fed on a ration of 25 grams of sterilized meat and 25 grams of polished rice daily. The rice used was the same as that used in Experiment 2 in which the fowls fed on 25 grams developed neuritis. The meat and egg were the same as that fed in Experiment 3, for Experiments 3 and 4 were performed at the same time, and the meat and egg were sterilized daily for both experiments so that there could be no possible difference between the meat and egg used in the two experiments. As a matter of convenience the birds were placed for the day in small individual coops containing a receptacle in which the weighed food was placed. The birds were then allowed to eat at pleasure, but at the end of the day any food remaining was fed by hand. During the first few weeks of the experiment the fowls ate well, and little remained to be fed by hand; but about the third week they lost appetite

and thereafter had to be fed largely by hand. From time to time individual fowls would appear to recover their appetites and for a few days would eat nearly all of their ration voluntarily. One bird never stopped eating and was practically never fed by hand. The results of this experiment are indicated in Table V.

TABLE V.

Fowl	Food	Neuritis	Remarks
1	egg and rice	none	Died in 40 days from forced feeding. No signs of neuritis
2	" "	"	Remained well for 111 days
3	" "	"	" " "
4	" "	"	" " "
1	meat and rice	41 days	None of the fowls in this group died. They developed complete paralysis of the legs, so that they were unable to stand, but some of them (1 and 3) made partial recoveries while still eating the same diet. No. 4 remained well 111 days, when the experiment was discontinued
2	" "	85 "	
3	" "	91 "	
4	" "	none	

As none of the birds in the group fed on egg and rice developed neuritis, it was necessary to show that it was not impossible for these particular birds to succumb to this disease. They were therefore placed on an exclusive diet of polished rice, and No. 2 developed neuritis 8 days later and died of the disease in 15 days, No. 3 developed neuritis in 19 days and No. 4 developed neuritis in 14 days.

In a similar way the fowls in the group fed on meat and rice were also placed on an exclusive diet of polished rice at the end of 111 days when the experiment was concluded. No. 1 died of neuritis in 8 days, No. 2 in 17 days, No. 3 in 15 days and No. 4 in 20 days.

This experiment indicates decidedly that when fowls are fed on sterilized meat or sterilized egg plus an equal quantity of rice, they do not develop neuritis any faster than when fed on sterilized meat or sterilized egg alone. On the contrary the reverse was the case. The fowls fed on egg and rice did not develop neuritis at all while the experiment lasted; and the fowls fed on sterilized meat and rice not only did not develop the disease faster on the average than the fowls fed on sterilized meat alone, but on the contrary appeared to show some tendency to spontaneous recovery on this diet, and all of the birds in Experiment 4 ended the experiment in better condition than the birds in Experiment 3. We cannot conclude therefore that the addition of rice to an already deficient diet hastened the onset of the disease, as we would expect, were Funk correct in his view that the vitamine is essential in carbohydrate metabolism. On the contrary, the addition of rice, probably by affording a more balanced ration, actually deferred the onset of polyneuritis.

CONCLUSIONS.

1. Fowls fed on polished rice and allowed to eat *ad libitum*, consumed from 30 to 70 per cent. of their body weight, and from 1.4 to 3.6 per cent. of their body weight daily before the development of polyneuritis.

2. Under these conditions, the rapidity with which polyneuritis develops bears no relation to the amount of rice eaten, but depends upon the idiosyncrasy of the fowl to this deficiency.

3. Experiments performed to determine the relation of the antineuritic vitamine to carbohydrate metabolism by hand feeding birds with varying amounts of carbohydrate foods are fallacious if the amounts fed are too excessive or too minute. The birds receiving too much die from overfeeding and the birds receiving too little die of starvation.

4. Fowls fed on sterilized meat or sterilized egg will develop polyneuritis.

5. When fowls are fed on equal parts of sterilized meat and rice or sterilized egg and rice, they develop neuritis more slowly than when fed on sterilized egg or sterilized meat alone.

6. These experiments all indicate that the antineuritic vitamine is not concerned in carbohydrate metabolism.

DISCUSSION.

In prescribing a diet to prevent beriberi, it is believed that it is sufficient to ensure the consumption of such a quantity of antineuritic foodstuffs (beans, rice polishings, barley, etc.) as experience has indicated will prevent the disease, without any reference to variations in the amount of carbohydrate consumed.

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A CASE OF TUBERCULOSIS IN A RAT¹.

By G. E. BODKIN. B.A., DIPL. AGRIC. (CAMB.), F.Z.S., F.E.S.,
Government Economic Biologist, British Guiana.

IN April, 1917, while examining some rats (probably *M. decumanus*) in company with Mr L. D. Cleare, Jr., my assistant, for external and internal parasites, the lungs of a male specimen were observed to be in a highly diseased condition.

This particular rat whilst under ether was observed to die some time before the others. On commencing to open the thoracic cavity the condition of the lungs was at once observed to be abnormal; on further examination the whole of the left lung was found to have almost completely decomposed into a thick yellowish-green pus.

The right lung was not so badly affected but was of a dull greyish-yellow colour with red blotches. No other lesions were observed in the specimen. The skin and fur were normal though infested with mites and a number of the well-known plague flea (*Xenopsylla cheopis* Roth.). The intestines contained specimens of the Cestode *Hymenolepis diminuta* Rudolphi, a common parasite of rats in this country.

Smears of this pus were made and submitted to Dr F. G. Rose, the Government Bacteriologist. He kindly examined the slides and his report appears beneath.

I may add that these rats were captured in a house inhabited by an individual well known to be tubercular.

22nd October 1917.

Government Bacteriological Dept.
 GEORGETOWN, DEMERARA.

In April, 1917, Mr G. E. Bodkin, Government Entomologist, brought me some smears taken from the lungs of a rat. These smears contained many pus-cells, broken-down lung-tissue and slender rod-shaped bacilli, some showing 'beading.' Gram-positive and acid-fast, in fact, morphologically identical with tubercle bacillus.

¹ Published by permission of the Director of Science and Agriculture, British Guiana.

Dean (1905, *Journ. of Hygiene*, v. 99) has described a leprosy-like disease in rats, but the clinical appearances as described by Mr Bodkin are quite unlike those of 'Rat-leprosy,' while the grouping and number of the bacilli did not in the least resemble those of the lepra bacillus.

There is therefore very little doubt in my mind that the bacilli were tubercle bacilli.

F. G. ROSE, B.A., M.B. (Cantab.)

Government Bacteriologist,

BRITISH GUIANA.

NOTE ON BODKIN'S PAPER, ON TUBERCULOSIS IN A RAT¹.

By LOUIS COBBETT, M.D.

This may have been an instance of avian tuberculosis in the rat, of which cases have been recorded by Max Koch and Rabinowitsch, though, so far as I am aware, destructive lesions in the lungs of this animal have not hitherto been described.

M. Koch and Rabinowitsch (1907)² examined fifty wild "grey" (? brown) rats, caught in poultry yards and pheasant preserves, and found six of them tuberculous. The lesions consisted of enlargement of the lymphatic glands, especially the mesenteric, and isolated nodules scattered in various organs. From two of these cultures tubercle bacilli were obtained and proved, on adequate investigation, to belong to the avian type. From this it has been concluded that the rat, like the mouse, sometimes contracts avian tuberculosis and may perhaps play a part in the transmission of the disease from one poultry yard to another.

On the other hand numerous experiments made on the tame "white" rat by the late Royal Commission on Tuberculosis showed this animal to be highly resistant to infection with all three types of tubercle bacilli. Even daily feeding for many months on the bodies of tuberculous guinea-pigs and tuberculous milk failed to produce any serious infection. Intraperitoneal injection alone proved fatal and that too only when enormous quantities of tubercle bacilli (10 to 50 m.g., enough to kill a calf) were introduced. Under these circumstances the rats developed a disease which progressed very slowly, and eventually died—it might be a year or more later. On macroscopic inspection lesions were very

¹ Kindly written at the request of the Editors.—G. H. F. N.

² M. Koch and L. Rabinowitsch (1907), Die Tuberkulose der Vögel. *Virchow's Archiv*, Beiheft 2, Vol. CXL, 246 [see p. 368].

inconspicuous, and nothing like cavitation was ever seen in the lungs, but on microscopic examination of crushed pieces of organ it is hardly too much to say that the tissues of the principal organs appeared to have been replaced by bacilli. Doses of 1.0 m.g. did not produce progressive disease¹.

The resistance of the white rat to artificial infection with tuberculosis, especially by feeding, was thus shown to be high, and it is difficult to understand how this animal can become infected naturally. That the brown species (*M. decumanus*) does so we have seen already, but there are no recorded instances known to me of natural tuberculosis in the wild black rat (*M. rattus*) or its near relative the tame "white" rat, nor is anything known about the capacity for resistance to artificial infection of the brown rat. If we may trust certain early experiments of Robert Koch² with various species of mice the field mouse is much more susceptible to infection with (mammalian) tubercle bacilli than the white mouse, and it is not unreasonable to suspect that there may be a similar difference of susceptibility in the brown and white rat. But about this we have no information, and even Koch's experiments need to be confirmed under more modern conditions.

¹ L. Cobbett, *The Causes of Tuberculosis*, p. 443.

² R. Koch, *Die Aetiologie der Tuberkulose*. Eng. trans. in *Microparasites in Disease*, New Syd. Soc. pp. 164 *et seq.*

SOME PROTEOLYTIC ANAEROBES ISOLATED FROM SEPTIC WOUNDS.

By HILDA HEMPL,

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(With 2 Text-figures.)

DURING four months' work with anaerobes, which were derived from wounds, a number of species have been noticed which appear to be hitherto undescribed. Two of these are proteolytic organisms and are not pathogenic to guinea-pigs when administered in large doses.

Owing to the extreme difficulty of getting pure cultures of anaerobes with ordinary technique, isolations were attempted by Miss M. Robertson and myself by means of Barber's method (*Philippine Journ. of Science*, vol. B ix. 1914, p. 307). Our results were very satisfactory. Instead of plating, isolations were made at random from the mixed cultures, some tubes receiving one bacillus and some several. The resulting cultures, if of interest, were treated in a similar manner. The type strains used for these descriptions were, to the best of my knowledge, derived from single bacilli isolated from apparently pure cultures, and they have behaved consistently in my hands.

ORGANISM I.

The first bacillus to be described has been isolated from two cultures. One case was that of a shrapnel wound of the maxilla which exuded a quantity of thick yellow pus. The sample was taken 20 days after the man was wounded and contained also *Bacillus sporogenes* (Metch.) and cocci. The second culture was taken from a pus wound located in the region of a tibia fractured by a high explosive shell. The sample was obtained 72 days after the injury occurred, and the wound had been treated with lotio rubra, permanganate, eusol, peroxide and saline. I found besides the organism in question cocci and end-sporing organisms with circular end-spores and bacilli of the morphology of *B. sporogenes* (both the ordinary variety and a smooth colonied variety) and of the morphology of *B. Hibler* IX. The organism described below was the

dominant one in both cultures; two or three others showed, when old, forms morphologically similar to it. The type strain was derived from a single organism isolated in turn from a single bacillus culture from a glucose agar tube colony.

STAINING and MORPHOLOGY. The bacillus is usually 3—6 times as long as it is wide and it has rounded ends. It may or may not retain Gram's stain. Under uniform conditions it is consistently paler and more gram-negative than *B. sporogenes*. The spores are oval and sub-terminal, though a few forms are so short as to be clostridial. Sometimes in meat cultures forms with a spore at either end are common. Gram's stain is more easily washed out from the body of the bacillus than from the sporing end. The organism forms spores readily on most media. On agar plates uniform gram-negative bacilli are the rule. In old meat cultures free-swimming forms disappear, but the mass of the meat is



Fig. 1. Representative forms from a two day meat culture.

inhabited by enormous pale gram-negative bacilli slightly tapering at the tip and bent in wide curves. This form is the most characteristic one of the species; similar forms occur on Dorset's egg medium. On gelatine it may grow as uniform gram-negative bacilli with pointed ends, spores being absent.

MOTILITY. The organism is motile but not highly so.

CULTURAL CHARACTERS. *Meat*¹. The growth on autoclaved meat is very characteristic. Twenty-four hours after inoculation there is usually heavy growth, the meat is pink, and some gas is present; the reaction is slightly acid. On re-incubation the meat settles more and more together with slight digestion, but it never disintegrates and never blackens. When pressed with a platinum loop it is soft and easily broken, but not

¹ Autoclaved meat medium and alkaline egg medium are described by Miss Robertson in her paper, "Notes upon Certain Anaerobes derived from Wounds," *Journ. of Pathol. and Bact.* xx. 348.

pasty and not finely granular. On long incubation it becomes bright terra cotta in colour, sometimes with a faded grey layer on top. Blackening, the general assumption of a brown or grey colour, disintegrating digestion or a refusal to digest at all are indications of impurity; on the other hand, several varieties of contaminating organisms may be present without greatly affecting the appearance of the meat. There is a putrefactive odour which is not especially characteristic.

Milk. In a hydrogen cylinder the litmus in milk is reduced if the organism grows; the milk is clotted *without* acid and slowly forms a soft irregular mass not torn by gas. Digestion of the clot begins after a few days and proceeds rather slowly; it is apparent from the yellowing of the liquid around the clot. This peculiar reaction has been consistent during long handling of the organism. The clotted milk placed on faintly blue litmus paper fails to turn it pink, and it is probable that the clotting is due to a weak rennet-like enzyme.

Alkaline egg. A fine coagulum is formed which renders the medium opaque.

Gelatine. The behaviour on gelatine, incubated at 37°, is rather irregular. Pure strains usually liquefy gelatine in two or three days although some gelatine tubes have remained unliquefied for two weeks. This irregularity is probably due to a difference in the quantity of the sowing used for the inoculation. There is a very misleading stage when a flake-like growth pervades the medium which stiffens as usual on cooling. On re-incubation for 24 hours the gelatine is liquefied.

Inspissated serum is attacked vigorously. Pitting occurs and the bases of the pits are white when many organisms are present. The surface becomes iridescent and sometimes brownish. Digestion is rapid at first but ceases before the medium is completely liquefied.

Dorset's egg medium is rendered soft and is cracked at the edges without liquefaction.

Ordinary broth and *glucose broth* are unsatisfactory media for this organism.

Action on carbohydrates. The organism was inoculated on serum litmus agar slopes containing various carbohydrates. It grew very well but failed to produce acid or gas in any case. The litmus was reduced and the mass of serum agar became somewhat pitted where the inoculation was the heaviest. The carbohydrates used were dulcitol, inulin, glycerine, mannitol, sucrose, glucose, maltose, laevulose and lactose. The strain used in these determinations was not the "single bacillus" strain.

Deep glucose agar tubes were excellent for the preliminary isolation of this organism. The colony in deep agar is at first round; later it takes the shape of a little worm, never growing more than 1·5 millimetres in length, and it is never woolly or radiate.

Growth on plates. This organism is easily grown on glucose agar plates. They should be dried for 20—40 minutes in the incubator before they are inoculated, and they must be exhausted and incubated immediately after inoculation. Colonies appear in 48 hours and at this period they are water-clear and very tiny. They are irregular in outline with a definite concentric ring arrangement, the middle portion being slightly granular under the microscope and the outer portion translucent. If the agar is too wet the growth will spread over the plate with fucus shaped projections.

After four days' incubation the colonies are easily visible through the substrate. They may be as much as two millimetres in diameter, are bluish grey in colour, and look to the naked eye as though they were speckled with white. Microscopically they are irregular edged and are no longer clear but are granular and wavy in texture.

Finally, these colonies are never woolly and never have fine processes; they are always minute and rarely round. They do not dig into the substrate nor are they raised or boss-like. They do not show up on the first day nor are they white or yellow.

PATHOGENICITY. Two cubic centimetres of a two days' broth culture of this organism failed to cause more than a slight swelling when injected intramuscularly into a guinea-pig.

TEMPERATURE OF GROWTH. This organism grows excellently at 37° and well also at 27° but at the latter temperature it does not start to grow as quickly. A newly inoculated meat tube shows no change at room temperature (*c.* 20°), but an old one goes on settling and reddening.

AGGLUTINATION. *Bacillus I* does not agglutinate with the anti-sporogenes serum of Weinberg.

Morphologically this organism would be easily confounded with *B. sporogenes* which it much resembles. The appearance of a young culture in meat medium resembles that of the acid producers, especially that of *B. perfringens*, but the organism bears so little morphological resemblance to any of those with which we are familiar that there is no danger of confusion.

ORGANISM II.

This is a typical proteolytic organism which is capable of splitting some of the single sugars as well as proteins. It has been isolated from

two cultures in this laboratory. One was taken from a very bad case of gas gangrene the material being sent from France by Capt. J. W. McNee, R.A.M.C.

The wound culture contained cocci, anaerobic bacilli of the morphology of *B. sporogenes*, *B. perfringens*, *B. Hibler* IX, an organism with circular end-spores and three apparently undescribed organisms. Organism II was first cultivated by Miss Robertson and handed over to me to describe. It was also isolated by myself from a culture from a bad gas gangrene case of Dr Brookes in the Horton War hospital. The infected leg was amputated and the patient ultimately recovered.

The other organisms found in this wound were cocci and anaerobes of the morphology of *B. perfringens*, *B. Hibler* IX, *B. sporogenes* and end-sporing organisms with circular end-spores. The strain used for this description was obtained from Capt. McNee's case and was derived from an apparently pure culture from a colony on an agar plate. Two bacilli isolated from this culture by Barber's technique gave a pure culture from which in turn a single bacillus was isolated for the type strain.

STAINING and MORPHOLOGY. The organism is more uniform in its morphology than most anaerobes. In the non-sporing state, the bacillus resembles exactly *B. perfringens*, being chunky and having very square ends. It takes Gram's stains so deeply that it usually appears black; however in a preparation from an agar slope many pale forms are seen among the dark ones. A young culture always contains many sporing individuals; after four days these may be no longer in evidence. The spores are usually central but they may be terminal. They are large in proportion to the size of the bacillus and usually markedly square at the ends. They do not bulge the sides of the bacillus to any appreciable extent and are usually clear and definite in outline. Two spores are never found in one bacillus. The size of the organism varies greatly; in a fresh meat culture the bacilli are all very large; a four days' culture has large and small forms. On broth, alkaline egg, serum and gelatine the bacilli are all much smaller than on meat. In old meat cultures and in old serum cultures long thick crooked gram-positive rods occur which are often granular.

MOTILITY. The organism is non-motile. .

CULTURAL CHARACTERS. *Meat.* The reaction on this medium is very characteristic. The meat retains the colour and form that it had before inoculation but it settles somewhat in the tube. A little gas forms in the first hours and then disappears; it is usually not noticed at all. When pressed with the loop the meat in old cultures is easily disinte-

grated but is not granular or brittle. It never becomes really digested and does not spontaneously disintegrate. The liquid above the meat is yellow and perfectly clear. A putrefactive odour is noticeable.

Milk. This organism does not clot milk or render it acid. It deposits the casein in microscopic granules and then digests it very rapidly, three days' incubation being sufficient to destroy the casein. The litmus is reduced.

Alkaline egg. A very soft coagulum is formed.

Gelatine is liquefied in less than 24 hours.

Inspissated serum. The growth on this medium is fairly plentiful. Digestion begins after a few days' incubation but stops before it has proceeded far owing to the fact that the organism ceases to grow.

Ordinary broth is a good medium.

Action on carbohydrates. A series of serum litmus agar slopes containing various carbohydrates were inoculated and the organism grew

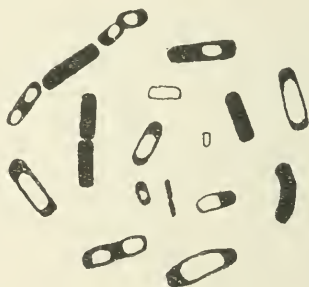


Fig. 2. Representative forms from a two day meat culture.

well. It produced both acid and gas on glucose, maltose and laevulose media, and acid alone on glycerine medium. Neither acid nor gas was produced on dulcitol, inulin, mannitol, sucrose or lactose media.

On *glucose agar plates*, treated as described for the preceding organism, the growth was very abundant. Pale bluish colonies were formed, usually irregular in outline but with smooth edges. The colonies were entirely homogeneous and of even thickness. The largest ones were two and three millimetres in diameter.

In *deep glucose agar tubes* tiny colonies are formed; they are irregular in outline but they are not radiate nor worm-shaped nor woolly. After five days' incubation they average $\frac{1}{2}$ millimetre in diameter and none of them is more than $\frac{3}{4}$ millimetre in diameter.

PATHOGENICITY. 2 c.cm. of a 48-hour broth culture failed to produce any pathological symptoms in a guinea-pig when injected into the muscles of the thigh.

TEMPERATURE OF GROWTH. The organism will grow excellently at 37° and also at c. 27° but is rather slow in starting to grow at the latter temperature.

AGGLUTINATION. Bacillus II does not agglutinate with the anti-sporogenes serum of Weinberg.

It is of interest to note that the organism bears a close morphological resemblance to *B. perfringens*. Some of its sporing forms are microscopically almost indistinguishable from a violently pathogenic gas forming organism isolated by the author from a human case. This pathogenic organism corresponds closely to vibron septique, Pasteur.

IDENTIFICATION. I think it is probable that Bacillus II is the same organism as *B. bifermentans sporogenes* of Tissier and Martelly (*Ann. de l'Institut Past.* 1902, p. 894). These authors say however that *B. bifermentans sporogenes* resembles *B. perfringens* and that their rôle seems to be identical. Whether this statement is due to a misunderstanding of the rôle of *B. perfringens*, I do not know, but I can draw no parallel between my organism and *B. perfringens* except the morphological one and the fact that they both split certain sugars. The putrefactive action of *B. bifermentans sporogenes* is far less than that of organism II, the former liquefying gelatine in a month the latter in a night; the former first changing the appearance of milk after five days, the latter showing digestion after a few hours and completely destroying the casein in a few days. These differences are perhaps to be accounted for by different media and technique. Recent experience with anaerobes inclines me to suggest that the strains of Tissier and Martelly were composed of the same organism as mine plus a small proportion of a member of the acid forming group which decreased the proteolytic power of the predominant organism.

Bacillus II resembles morphologically *B. sporogenes foetidus* of Choukewitch (*Ann. de l'Inst. Past.* March and April, 1911) but the latter organism is feebly motile and does not form gas in deep glucose agar.

Clostridium foetidum of Liborius (*Zeitschr. f. Hyg.* 1. 1886, p. 160) and *Clostridium foetidum cernis* of Salus (*Archiv f. Hyg.* LI. 118) do not resemble organism II morphologically and they are highly motile.

The "pseudo-oedem bacillus" of Liborius is very meagrely described; it somewhat resembles my organism but is said to form at times two spores in one bacillus.

I wish in conclusion to express my heartiest thanks to Miss Muriel Robertson for her interest in this work and to Dr Harden of the Lister Institute for so kindly affording me the courtesies of the laboratory.

THE SOURCES OF INFECTION IN FOOD POISONING OUTBREAKS.

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COMPARED with 20, or even 10, years ago our present knowledge of food poisoning outbreaks is extensive and in certain directions fairly complete. In spite of this greatly extended knowledge there are some aspects in regard to which we are yet lacking in fundamental information. This is particularly the case as to the precise sources of infection. It may be accepted as a demonstrated fact that most outbreaks of food poisoning are due to infection of the food eaten with one or other member of the Gaertner group of bacilli. The present paper is only concerned with the outbreaks associated with this group of organisms. A study of the individual outbreaks usually supplies evidence which definitely incriminates a certain article of food, and for most of the recent outbreaks further evidence is forthcoming that this has been infected with one or other member of the Gaertner group of bacilli. Tracing the matter a step further back it is only in a quite small minority of outbreaks that the recorded facts show how the food has become so infected. In a proportion of cases, perhaps more than half for continental recorded outbreaks but in only a small fraction of the British outbreaks, it is true that definite evidence is forthcoming showing that the meat was derived from an animal itself suffering from general or local disease caused by Gaertner group bacilli. Even, however, for these cases our recorded knowledge ceases with this information, and we do not know how these animals became infected or whether they represent isolated cases or are part of widespread epidemics amongst the animals affected.

For the majority of the outbreaks, i.e. those in which no animal affected with disease is reported, our information as to causation is absolutely negative. Our ignorance on these points is really extraordinary and not less so is the complacency with which these important questions are ignored in the majority of the reported outbreaks. I am of

opinion that the widespread, but inaccurate, idea that these organisms are ordinary inhabitants of the animal intestine is frequently the cause of the failure to probe deeper into the precise channels and methods of infection.

Three hypotheses may be advanced to explain the origin of the Gaertner group bacilli in those cases for which definite disease of the animal supplying the meat could not be traced. These three hypotheses are fully discussed in my Report to the Local Government Board on Food Poisoning and Food Infections (Savage, 1913) and need only be mentioned here.

The first view suggests that the bacilli are of human origin, the food being infected with pathogenic Gaertner bacilli from a human source, i.e. a case of disease (paratyphoid fever) or a carrier case. In my opinion the available evidence certainly excludes this conception.

The second hypothesis, that the Gaertner group bacilli which set up the food-poisoning outbreaks are derived from ordinary faecal infection of the food, is contrary to ascertained fact, as the extended investigations which have been carried out in this country show conclusively that this group of organisms are not natural inhabitants of the intestine of the domestic animals used for food.

The remaining hypothesis is the one which I have advanced elsewhere and which, I believe, best explains the available facts. This hypothesis suggests that the Gaertner caused food-poisoning outbreaks are due to infection of the food with virulent Gaertner group organisms, derived either from animals which are at the time suffering from disease due to Gaertner group bacilli or from animals acting as carriers of these bacilli.

It will be noted that this hypothesis is adequate to explain the outbreaks associated with meat derived from a definitely diseased animal, and also those in which infection occurs during the preparation or storage of the food for consumption. In the above mentioned Report I have given data which make this view a probable one. The purport of the present paper is to further elaborate the significance of this hypothesis and the lines by which its truth can be tested experimentally.

If this view is accepted it implies that certain of the animals used for human food, or which come into contact with food eaten by man, suffer from Gaertner diseases and that in this way the food becomes infected and originates a human outbreak. We should expect therefore to find that diseases of this causation were recognised amongst the domestic animals.

ANIMAL DISEASES CAUSED BY INFECTION WITH MEMBERS
OF THE GAERTNER GROUP OF BACILLI.

1. *Swine Fever*. This disease is now generally accepted as due to a filterable virus, but in a considerable proportion of cases *B. suipestifer*, a member of the Gaertner group, is also found to be present, not as a mere passive concomitant but apparently with a distinct, although subsidiary, disease-producing rôle. The proportion of cases of swine fever in which this bacillus is found seems to vary from 0 to 45 per cent. or over (Uhlenhuth, Hübener, Xylander and Bohtz (1908), Uhlenhuth and Haendel (1913), Grabert (1907)).

While recorded investigations give the occurrence of this bacillus as prevalent to the considerable extent indicated it is of importance from the present point of view to point out that in many of the records the term *B. suipestifer* is used in a wide sense and include many organisms which with any strict definition of the Gaertner group, such as should be employed, must be excluded as not members of it. Uhlenhuth and Haendel (1913) discuss at some length the varieties of *B. suipestifer* and while they point out that many of these are culturally identical and differ chiefly as regards virulence, motility and other variable characters, yet they include under this term as varieties organisms described by Dorset and also by Joest and Grabert which do not ferment glucose and the bacillus described by Rietsch and Jobert from swine fever pigs at Marseilles which produced acid and clot in milk. Also the eight bacterial strains described by Uhlenhuth, Hübener, Xylander and Bohtz (1908) which failed to be agglutinated must be regarded as suspicious, although they report them as culturally in agreement with *B. suipestifer*, since they do not appear to have recognised the existence of the para-Gaertner organisms which I have described (Savage, 1912). (The dulcitate and salicin tests for example were not employed.)

Certain named varieties of *B. suipestifer*, i.e. *B. typhi suis* (of von Glässer) and *B. suipestifer voldagsen* show, in at least some strains, cultural differences from true *B. suipestifer*.

Two recent investigations in Scotland and America respectively are of interest in this connection. In Scotland M'Gowan (1915) carried out some very thorough post-mortem investigations on cases of swine fever. Cultures were made from the various organs and lesions of 11 cases of this disease in the neighbourhood of Edinburgh and 27 organisms were isolated. None of them however belong to the true Gaertner group as shown by their action upon glucose, dulcitate and litmus milk, while all

(with one possible exception) were non-motile. In two other isolated cases true *B. suispestifer* was not found.

Ebersson (1915) studied the chief groups of organisms found in a number of hogs artificially infected with the hog cholera virus, 55 animals being examined: 106 so-called paratyphoid strains were isolated. The cultural character of these organisms are not given in detail, but for some the few cultural tests employed show clearly that they are not true Gaertner strains while for the rest the tests employed are totally inadequate to say if they are Gaertner organisms or not. Yet in this paper it is set out that "classification of the organisms shows that the greatest number belonged to the paratyphosus B. group."

In a recently issued paper Jordan (1917) has shown that the majority of the *B. suispestifer* strains tested by him, and which were mostly derived from affected pigs in America, showed cultural differences (i.e. attack arabinose and dulcitol slowly or not at all) from those given by the strains isolated from food poisoning outbreaks.

It is I think a fair and justifiable criticism to make that if more exact and extended cultural investigations were made of the organisms present in pigs suffering from swine fever a considerably smaller percentage would be recorded as true *B. suispestifer* strains. Apart however from such necessary corrections it would still appear to be true that genuine Gaertner organisms are frequently met with in cases of swine fever, that is organisms which with the most refined bacteriological tests are indistinguishable from the strains isolated from human victims of food poisoning.

2. *Septicaemia and other diseases of calves.* It would appear that while calf septicaemia, dysentery and pneumonia are caused by a number of different bacteria, in a certain proportion of cases Gaertner group bacilli are either the cause or are found to be present.

Thomassen (1897) described a fatal septicaemia in calves in the neighbourhood of Utrecht and isolated a bacillus, now identified with *B. enteritidis*, from the spleens, kidneys and other organs of the affected animals. Since that date Gaertner group bacilli have been reported by several continental observers in cases of septicaemia, white scour, etc. Such reports have been published by Zeller (1909), Titze and Weichel (1909), Schmidt (1908), Riemer (1908) and Winzer (1911). The necrotic areas sometimes met with in the spleen, liver and other organs of calves in slaughter houses have been shown to sometimes contain Gaertner group bacilli (see for example Joest (1914)).

Uhlenhuth and Hübener have shown that Jensen's paracolon bacillus,

described by him as the cause of calf dysentery, is, in at least many cases, a true Gaertner group bacillus. Jensen's (1913) own figures however show that Gaertner group infections are only responsible for a small proportion of the cases which he groups under the term "Kälberruhr." Of 251 cases he only found these bacilli in 16 or 6·4 per cent. Apparently they were all in new born calves and no doubt were all infected at parturition. It is possible that a few may be of human origin but this is a most unlikely source of infection, and the most probable origin is either an infected cow, infected litter, etc.

That bacilli in these calf infections may be pathogenic to man is illustrated by a case recorded by Meyer (1916). A man 26 years old became infected by feeding with a Gaertner group bacillus isolated from the heart blood of a calf which had died from infectious diarrhoea. The patient suffered from severe abdominal cramps, nausea, diarrhoea, flatulence and elevation of temperature but recovered within a week. He had been feeding a calf with milk containing this bacillus. A bacillus was recovered from his excreta identical in every particular with this organism, while specific agglutinins developed in the blood.

3. *Pyæmic and septicæmic conditions in the domestic animals generally.* Bollinger in 1876 first drew attention to the frequency of the association of food poisoning outbreaks in man with the consumption of the meat of animals suffering from such diseases. In a certain number of cases Gaertner group bacilli have been isolated from such conditions and apart from food poisoning outbreaks. For example the *B. morbificans bovis* of Basenau is a Gaertner organism and was isolated by him from a cow emergency slaughtered on account of puerperal metritis, while Fisher in 1896 isolated *B. enteritidis* from the spleen of a cow with udder inflammation. Also of particular interest is the outbreak of acute mastitis in cows recorded by Zwick (1909) and Zwick and Weichel (1910) in which Gaertner group bacilli were isolated from two out of 21 cases.

Against these recorded cases we have the fact that a long series of bacteriological examinations of animals suffering from septicæmic diseases—made on the continent—have failed to show Gaertner group bacilli, except in rare instances.

We must therefore conclude that while conditions of this sort may be caused by Gaertner group bacilli it is a rare and exceptional occurrence and the vast proportion of cases are due to the ordinary pathogenic pyogenic bacteria.

4. *Enteritis in cows.* Such cases are of exceptional interest in view of the fact that in a number of meat poisoning outbreaks the meat has

been derived from a cow suffering from enteritis. Instances of enteritis in cows, unassociated with food poisoning outbreaks, in which Gaertner group bacilli have been isolated are rare and I have only been able to find the following.

Mohler and Buckley (1902) record an outbreak in which seven out of 21 cows in a cowshed suffered from enteritis and died, while three others exhibited early symptoms but recovered. A Gaertner group bacillus was isolated from all the fatal cases. One cow apparently recovered from the acute attack, but ultimately died 26 days after the onset.

Meissner and Kohlstock (1912) describe an interesting outbreak. Dysentery was prevalent amongst some calves causing the death of some, although the majority recovered. One of these animals which had apparently recovered was transferred to pasture land shared by a number of cows. A number of these then suffered from diarrhoea and enteritis and died. The only one investigated showed *B. enteritidis* in pure culture. The affected cows were then moved to cow stalls. These contained two lame cows and a 1½ year old bull none of which had been out in the pasture field. One of these two cows fell ill after the addition of the affected cows and from it *B. enteritidis* was isolated. The calf, which had apparently recovered and which was transferred to the field, was then examined bacteriologically and *B. enteritidis* isolated (the report does not say from which organs). A further calf which had died of enteritis was also subsequently examined and *B. enteritidis* isolated.

In this outbreak we have an illustration of a calf suffering from a Gaertner group infection recovering and acting as a carrier of infection.

5. *Abortion in mares.* While it is evident in the great majority of cases that this condition, at least in this country, is due to bacteria other than food poisoning bacilli (see *Annual Report* (1914) of Chief Veterinary Officer of the Board of Agriculture) there is evidence that in certain outbreaks bacilli of the Gaertner group are present in the lesions, and in a number of cases have been reported as the cause of the condition. In 1893 Kilborne and Smith (U.S. Board of Agriculture, 1893) studied an abortion outbreak occurring amongst the mares of a large stud in Pennsylvania and isolated a bacillus which they grouped as a hog-cholera bacillus. The characters described are insufficient to group it as undeniably a true Gaertner group organism, but it fermented glucose but not lactose and saccharose, and the other characteristics given are those of Gaertner organisms.

Similar bacilli have been isolated from American outbreaks by Good and by Meyer and Boerner (1913). The latter observers from an outbreak in 1913 in Pennsylvania isolated a bacillus which they called *B. abortus equi*. The serum of the aborting animals gave positive agglutination results as high as 1 : 2500 with this bacillus, but a complete complement fixation with comparative high titres was only noted in four animals. This bacillus exhibited the cultural characters of the Gaertner strains except that the growth on agar slope was membranous, dry and brittle, and the gas produced in dulcitate media was large in amount. One of the two strains isolated by Good also exhibited similar characteristics on agar. Their agglutination results suggest that their *B. abortus equi* is neither *B. enteritidis* nor *B. paratyphosus* B, while it was only partially in agreement with the only strain of *B. suispestifer* tested. The authors put it in a separate sub-group.

In 1897 Lignières and in 1905 Lignières and Zabala isolated a Gaertner group bacillus from a series of cases of epizootic abortion in mares, sheep and cows in France and Argentina. In Holland outbreaks ascribed to members of this group have been described by de Jong and by Van Heelsberger (1914). The bacillus isolated by Van Heelsberger was pathogenic to the smaller laboratory animals and, as far as its cultural characters were tested, agreed with the Gaertner group. The agglutination reactions seem to show that it is not identical with either *B. enteritidis*, *B. suispestifer*, or *B. paratyphosus* B.

I am not concerned with the question as to how far these bacilli were the true cause of the abortion and other symptoms or whether they played a rôle analogous to that of *B. suispestifer* in swine fever, but their presence in this condition in horses is certainly of considerable interest.

6. *Certain diseases of birds.* Epidemics, usually marked by a high fatality rate, have been recorded as affecting a number of different species of birds and from which Gaertner group bacilli have been isolated. The best known are the outbreaks in parrots, the so-called Psittacosis disease (see Baumgarten's *Jahresbericht*, 1896, for an account of several outbreaks). Nocard in 1893 isolated a bacillus, which he called *B. psittacosis*, from the bone marrow of birds which had died on the journey from Buenos Ayres. In subsequent epidemics this bacillus has been isolated both from the diseased parrots and from the blood of the human cases. From the parrots the disease has spread to man, and in April, 1892, an extensive outbreak occurred in Paris, with 42 known cases and 14 deaths. *B. psittacosis* is undoubtedly a Gaertner group organism and probably identical with *B. suispestifer* (Böhme, 1906, Selter, 1916).

Tartakowsky has described an infectious enteritis in sparrows due to a Gaertner bacillus.

Joest (1907) isolated a bacillus, apparently a Gaertner group organism, from a canary suffering, with others, from an epidemic disease associated with catarrhal enteritis and splenic tumour. Zingle (1914) in 1913 isolated in pure culture a Gaertner group organism from pigeons in an outbreak affecting 14 birds in the Military pigeon station at Strassburg. This strain was agglutinated nearly to the titre limit by a *B. paratyphosus* B serum but was only partially agglutinated by a *B. enteritidis* serum, so apparent is the former organism, according to German nomenclature. Manninger (1913) investigated three birds of the finch family sent to him from the Buda-Pest Zoological Gardens and isolated from them a Gaertner group bacillus. The birds suffered from an acute intestinal catarrh. Like the bacillus from the last outbreak this organism was only agglutinated in moderate degree by a *B. enteritidis* serum but to the titre limit by a *B. paratyphosus* B. serum.

The outbreak of acute infectious disease in young pheasants recorded by Klein (1893) in which over 700 out of 1800 died may have been due to a Gaertner strain, but the characters of the bacillus isolated from the heart blood are insufficient to settle this point and the fact that indol is said to have been produced is against this assumption.

7. *Canine distemper*. I have only come across one report dealing with the presence of Gaertner bacilli in dogs. Torry and Rahe (1912) in a series of 63 consecutive cases of natural and experimental distemper isolated *B. enteritidis* in one or more of the internal organs in 12 cases (19 per cent.). They suggest the bacilli invaded the organs in the final stages since 75 per cent. of the findings were in animals severely attacked. The bacilli were non-toxic to dogs. Their characters are not given in detail but were said to be identical in cultural and agglutination characters with *B. enteritidis* (Gaertner).

8. *Diseases amongst rodents*. *B. typhi murium* (a Gaertner group organism) was isolated by Löffler as the cause of an epidemic in mice and has been subsequently isolated from other mice epidemics. It has been used as living poison to set up an epizootic among mice and so cause their extermination. In the same way a number of Gaertner group bacilli have been used to set up infective disease in rats. Of these Danysz's bacillus is the best known.

Spontaneous outbreaks of infectious disease amongst rats and mice and due to Gaertner group strains are not uncommon. Three such have occurred at widely different periods amongst my own laboratory mice.

while a number of outbreaks abroad have been recorded. These bacilli have occasionally been found in rats and mice not showing definite disease (Savage and Read, 1913). The question of the infection of rats and mice is obviously of considerable practical importance in view of the frequency with which these animals gain access to food used for man and animals.

It is now well recognised that persons suffering from typhoid fever and paratyphoid fever after recovery may continue to excrete the bacilli of these diseases for prolonged periods and in this way may act as carriers of infection. It is highly probable therefore that animals suffering from Gaertner group infections, caused by bacilli closely allied to those responsible for these two diseases, may after recovery also act as carriers of infection. Information in regard to this very important point and as to the duration of the carrier stage is most desirable and it is unfortunate that recorded data are so scanty. Some ascertained facts are however available.

O'Brien (1910) recorded a naturally occurring outbreak caused by a Gaertner group organism (*B. suispestifer vel B. aertryche*) amongst the laboratory stock of guinea-pigs, only 21 out of 500 surviving. Examination of the faeces of nine of the survivors showed that five animals were carriers of the bacillus. The serum of four of these agglutinated the bacillus in dilutions of 1 : 50 and 1 : 100. The duration of the carrier state was not worked out but these five excreted the bacilli intermittently five months after the epidemic.

Petrie and O'Brien (1910) also studied the excretion of bacilli in feeding experiments and record "in the course of a series of feeding experiments we have found that guinea-pigs fed with cultures may excrete the bacillus in the faeces for some time subsequently while remaining apparently healthy, and that the blood of some of these animals agglutinates the bacillus."

A fact which is very obvious from the above summary of diseases in animals caused by Gaertner group bacilli is that, apart from outbreaks in rats and mice and cases of swine fever, all have been described abroad. I have been unable to find any reports of similar infections due to these bacilli in Great Britain. Are we to assume they do not exist?

In favour of such a supposition is the fact that the rigid delimitation of the Gaertner group to organisms with certain definite characters, which has been adopted by most workers in this country, does not prevail to the same extent in Germany and other parts of the continent, and possibly not all these recorded outbreaks are due to true Gaertner group

organisms. A careful study of the original papers however makes this improbable for more than a minority.

A study of the food poisoning outbreaks recorded in this country is most disappointing from this point of view since so few of the records supply any information in regard to this most important matter. In only two of the 79 British outbreaks summarised in my Report to the Local Government Board is any evidence adduced as to the existence of disease in the animal supplying the incriminated food. Two others have since been described. Some particulars of these four cases are of interest.

In the Murrow outbreak (Savage and Gunson, 1908) amongst the bones, etc. used to make the brawn which caused the outbreak was a pig's foot which was obviously diseased and which, from the description available, probably had an abscess on it. The pig was sufficiently affected to have to be taken by cart to the place of slaughter.

In the Limerick outbreak (McWeeney, 1909) the available evidence is not very precise, but the infected meat (from an ox) was purchased ready killed by the contractor and at an unusually low price. No reliable information was obtainable as to the condition of the animal prior to or at the time of slaughter, but since the butcher who sold it to the contractor would appear to have sold it below cost price it is highly probable that it was not sound healthy meat.

In the Newcastle-upon-Tyne outbreak in 1913 (Kerr and Hutchens, 1914) due to infected milk derived from a cow, recently calved and added to the herd, which had shown signs of illness a day or two before, and died almost coincidently with the occurrence of the first cases of the outbreak. Although the milk had markedly diminished and was abnormal in character it had been mixed with that from the rest of the herd and sold. *B. enteritidis* was isolated from the internal organs, intestinal contents and from the milk, drawn from the udder after death, of this cow. No information was available as to how this cow became infected or if other animals had been attacked.

The fourth instance, one mentioned by Hutchens (1914) but not described in detail, is an outbreak of food poisoning affecting 105 persons after drinking milk, a Gaertner group bacillus being recovered from the milk on two consecutive days before the cow died. The cow in this case had also recently calved.

In contrast to the above I may mention that in 50 per cent. of the continental outbreaks summarised in the same Report definite disease of the animal supplying the food was found. Owing in part to the inadequate system of food inspection in this country it is often extremely

difficult to obtain reliable information as to the health of the animals supplying any particular specimen of meat.

In connection with infections with one or other member of the Gaertner group it is an important fact that naturally occurring outbreaks nearly always show a low rate of mortality. In human food poisoning the case-mortality rate for a large number of outbreaks was only 2.7 per cent.

With laboratory animals subcutaneous and especially intraperitoneal inoculation usually produces a fatal result, but it is far otherwise when natural methods of infection are employed. Feeding experiments both with the smaller laboratory animals and with larger animals such as dogs, calves and goats have given very irregular results, and this with strains showing evidence of high virulence when inoculated subcutaneously or intraperitoneally. In some of the animals fed in this way agglutinins developed, so probably some infection resulted. The following is an interesting instance.

Reinhardt and Seibold (1912) fed a goat with four agar cultures of a strain of *B. enteritidis* which had previously been passed through four other goats, causing marked illness in them. when the method of introduction was by intraperitoneal injection or injection into the udder or knee joint. Every day for the next five days the animals received by the mouth an emulsion containing the whole of a 24 hours old agar slope culture. Some rise of temperature was noted but there was no noticeable effect upon the health of the goat. Bacteriological examination of the blood was negative. Before feeding no agglutinins for this bacillus could be demonstrated and none four days after feeding. Nine days after feeding a reaction of 1 : 40 was obtained while 16 days after the start of the experiment the serum reacted to the bacillus in a dilution of 1 : 1280. The goat was killed 16 days after the start of the feeding. There were no pathological lesions while all the organs were sterile. Nothing is said as to the presence of the bacilli in the intestinal contents.

The same authors give an interesting instance of a case of natural infection which throws light upon the way these diseases may be spread. A goat was inoculated by the injection of an emulsion of four agar cults into the uterus this causing marked illness. The three kids of this goat born two days before the inoculation were removed from her but were brought back in the evenings and drank her milk. Two remained unaffected but the third sickened eight days after the mother was infected and died three days later showing, *post-mortem*, gastritis and duodenal catarrh. The bacillus was readily isolated from the internal organs.

It is of interest to note that recovery after severe infection may take place in naturally infected animals. In the two mastitis cases of Zwick and Weichel (1910) in one the cow had one quarter of the udder much swollen, hard and painful, yielding only a little yellow watery fluid with grey-white flakes from which *B. enteritidis* was isolated. The cow was "off its feed" and the temperature was raised. Five days after the onset the general condition improved while milk could be obtained from the affected quarter, but the next day the animal was worse with increased udder swelling and also ulceration. The animal then became rapidly better, the ulcers healed and the milk secretion gradually returned. Unfortunately the report does not say how long the bacilli continued to be excreted in the udder fluid or if the bacilli were excreted with the intestinal contents. Such a case might be a carrier of infection for a long period and a possible source of infection to man.

The necrotic foci sometimes found in the liver, spleen and kidney of apparently healthy animals and especially calves have in some instances been shown to contain Gaertner group bacilli (Langer, Ledschbor, Joest, etc.). They indicate recovery after infection.

Considerations of the facts detailed in this paper suggest certain conclusions of great practical importance in connection with the causation of food poisoning outbreaks.

Extended direct examination has proved that Gaertner group bacilli are not natural intestinal inhabitants either of man or of animals used for human food or which come into contact with food. The instances in which true Gaertner group bacilli have been found in such situations are rare and can be readily accounted for on the supposition that they are bacilli present as the result of previous infection (carrier state) and are strictly comparable to the presence of typhoid bacilli in the human intestine.

Gaertner group bacilli are pathogenic, but with much variation as to degree of pathogenicity, for most of the domestic animals and various investigations show that spontaneous outbreaks of disease, with however a number of different local manifestations, are set up by them in these animals.

The prevention of human outbreaks of food poisoning, and possibly a considerable amount of unrecognised disease in animals, can only be attained by an extension of knowledge as to the extent to which these pathogenic organisms are a cause of animal disease.

I desire to emphasise the need for exact investigation of the extent to which these bacilli are responsible as a cause of animal disease and

the fact that there appear to be no records of outbreaks of Gaertner infections in animals *in this country*, apart from cases traced back to animals because they had caused an outbreak of human disease. The investigation of these problems and questions is clearly a matter in which co-operation between the bacteriologist and the veterinary surgeon is required and such co-operation should yield results of great practical value.

The investigation of the bacteriology of cases amongst domestic animals of abortion, septic diseases, enteritis, metritis, etc. from the point of view of the presence or absence of members of the Gaertner group of organisms, either as the causal organism or as a concomitant bacillus, does not appear to have been undertaken in any systematic manner. I would suggest that extended observations on these lines with careful inquiry and following up of all cases which show the presence of members of this group of bacilli is likely to throw considerable light upon the causation of human food poisoning¹.

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¹ The importance of this line of inquiry has been apparent to me for a good many years and I have carried out a series of investigations from several points of view, some of the results of which I hope to publish shortly.

As regards the examination of material from diseased animals or from animals which have died from one of the diseases associated with food poisoning bacilli, I have been greatly hampered by inability to obtain suitable cases for examination.

I should be very glad to arrange for the bacteriological examination of such material and would be pleased to supply outfits and detailed particulars to any one in a position to obtain material of this nature.

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FURTHER INVESTIGATIONS UPON THE DISTRIBUTION OF GAERTNER GROUP BACILLI IN DOMESTIC AND OTHER ANIMALS.

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IN another paper I have set out in some detail the reasons which suggest that the sources of infection in food poisoning outbreaks must be sought primarily in infections of some of the domestic animals used for food and possibly in part from rats and mice which gain access to food. In the present report the results of a series of investigations in this direction are described, using the above hypothesis as a basis of inquiry. Much difficulty has arisen from inability to obtain sufficient suitable material for examination.

The investigations have been along the following lines:

I. Examination of internal organs of domestic animals slaughtered for food and presumably healthy.

II. Serological examination of the blood of healthy domestic animals for the presence of Gaertner group agglutinins.

III. Bacteriological and serological examinations of rats.

IV. Bacteriological examinations of domestic animals suffering from certain diseases.

I am much indebted to Dr Howarth, Medical Officer of Health, City of London, and to Mr T. Jones, Sanitary Inspector, Weston-super-Mare, for specimens from healthy animals killed in slaughter houses, to Dr R. A. O'Brien of the Wellcome Physiological Research Laboratories for sera from healthy animals, to Dr Walker Hall, Professor of Pathology, Bristol University, for specimens from rats, and to Sir Stewart Stockman for the organs from several diseased animals.

The investigations are to a considerable extent inter-dependent but are most clearly explained by separate presentation.

I. EXAMINATION OF INTERNAL ORGANS OF HEALTHY
DOMESTIC ANIMALS.

Theoretically all the chief internal organs should be examined, but practical experience shows that if any infection results from members of the Gaertner group of bacilli, and such bacilli are present in the internal organs (apart from the intestines), they will always be found in the spleen although not necessarily in the other internal organs. I know of no recorded cases in which such bacilli have been found in liver, kidney, marrow, etc. and have been absent from the spleen. It is therefore not of material importance to examine other organs in addition to the spleen, and for the most part this was not done.

The specimens were all obtained at Weston-super-Mare from animals killed in the Public Abattoir, passed as healthy and subsequently used for human food. The usual methods to avoid outside contamination were taken. The primary inoculations were direct upon two salicin lactose bile salt agar plates while also one agar plate was brushed. In addition a little of the tissue from the interior of the organ was added to mannite malachite green broth and this was brushed over lactose bile salt agar plates, if any growth occurred after 20 hours' incubation at 37° C.

Great care was taken to examine the organs in a fresh condition. The interval between slaughtering and examination was rarely over three to four hours and in no case was over six hours.

The spleens examined were 24 from healthy pigs (except that one was tuberculous) and 10 from healthy calves. All except one appeared perfectly healthy to the naked eye. The remaining spleen showed scattered through it a number of small nodules about $\frac{1}{8}$ inch across. They were hard and not caseous and further examination proved them to be tuberculous. They were associated with tuberculosis of the intestines.

Although *B. coli* and other organisms were found (see below) no Gaertner group bacilli were present in any of the samples. In only one case (Calf No. 16) were para-Gaertner bacilli found. With the cultural definitions for the Gaertner group frequently employed on the Continent this organism might easily be included as a member of that group and it is therefore of some interest.

Its essential characters are as follows: a short bacillus with rounded ends, very actively motile. Stains with ordinary stains but gram-negative. In litmus milk produces first a little acid but after 6—9 days at

37° C. becomes markedly alkaline. No indol formed in peptone water. Bluish translucent growth on gelatine slope, no liquefaction. Ferments, with gas production, glucose and mannite but not salicin, saccharose or lactose. In dulcitate produces acid but no gas.

This organism agrees therefore in all its cultural characters with the true Gaertner group bacilli except that it fails to produce gas in dulcitate, although acid is produced. Tested on several occasions and after different intervals it still failed to produce gas in dulcitate broth.

It was non-pathogenic to a mouse when injected subcutaneously. That it was a para-gaertner organism is also shown by the fact that it failed to be agglutinated in one per cent. dilution by *B. enteritidis*, *B. suispestifer* or *B. paratyphosus* B sera each with a titre of over 1 : 1000. The spleen of this calf was examined $2\frac{3}{4}$ hours after slaughter and was quite healthy and free from all necrotic areas to the naked eye. The serum of this calf failed to agglutinate any of the three sub-group organisms (*B. enteritidis*, *B. suispestifer*, *B. paratyphosus* B) even in a dilution of 1 to 20; while it also failed to agglutinate in the same dilution the para-Gaertner bacillus isolated from the spleen of this animal.

In a second series the gall bladder and bile and a piece of the small intestine were examined very carefully from eight pigs while in four further pigs a piece of large intestine was also examined. All were examined within a few hours of death. In no instance could either Gaertner or para-Gaertner group bacilli be isolated. In several of these cases the serum of the pigs showed well marked agglutination with one or other of the Gaertner strains (see section II).

Bacterial content of the spleens apart from Gaertner group organisms. Although not germane to the objects of these investigations the results are of sufficient interest to be briefly recorded.

Of the 24 spleens from pigs examined, 12 were sterile as regards aerobic organisms (no anaerobic cultivations were made). No special incubation of the whole organ was practised, as in Conradi's method, the inoculations being made from the interior of the spleen by rubbing a sterile glass rod into the pulp and then using this to inoculate the plates, while a small fragment was added to the malachite green broth.

In the 12 sterile cases no growth took place in the malachite green broth or upon the one agar and two lactose bile salt agar plates. The remaining 12 all showed bacteria. *B. coli* group organisms were present in nine, but in no instance in pure culture, being accompanied usually by non-lactose fermenting bacilli while streptococci were present in several and staphylococci in one or two other cases. Two of the remaining three

showed streptococci in pure culture and from the remaining spleen only non-lactose fermenting bacilli were obtained which slowly fermented glucose.

It is of interest to note that the interval between slaughter and bacteriological examination was not materially longer for the sterile spleens than for those containing bacteria. Stated in hours, this interval was for the sterile cases respectively 3, $1\frac{1}{2}$, $1\frac{1}{2}$, 6, 2, 2, 3, 4, 3, 4, 4, 4, hours:—an average of 3.17 hours. For the bacteria-holding spleens the interval was $2\frac{1}{2}$, $3\frac{1}{2}$, 6, 6, $4\frac{1}{2}$, 2, 3, 3, 3, 4, 4, $4\frac{1}{2}$ hours:—an average of 3.83 hours.

No evidence was available to show that the age of the animal had any relationship to the bacterial content of the spleens. It was difficult to ascertain the exact ages of the animals but in 17 cases this was fairly reliably obtainable. The approximate ages of seven of the pigs with sterile spleens were: 4, 8, 5, 5, 8, 10, 10 months—average 7 months and of eight non-sterile cases, 6, 4, 4, 12, 12, 12, 8, 5 months—average ages 7.9 months.

The spleens from the 10 calves were examined in exactly the same way. Of these five were sterile, five non-sterile. The organisms found in the five cases were *B. coli* group bacilli, white staphylococci, a few streptococci, a glucose fermenting, lactose non-fermenting bacillus and the para-Gaertner bacillus (in pure culture). The bacilli were in smaller numbers than in pig spleens while in most cases only one kind of bacterium was present in each specimen.

The interval between slaughter and examination was for the five sterile cases 6, 3, 2, 7, 4 hours respectively, an average of 4.4 hours and for the non-sterile 2, 3, 2, $2\frac{1}{2}$, $6\frac{1}{2}$ hours respectively, an average of 3.2 hours.

All the calves were under three months old. The greatest care was taken to avoid outside contamination and the inoculations were all made from the interior of the spleens.

The results show definitely that in 50 per cent. of the animals selected (pigs and calves) spleens from perfectly healthy animals examined within a few hours of slaughter showed bacilli. The want of correspondence between bacterial content and interval since death and the general shortness of the interval between slaughter and examination makes it a reliable deduction that such bacilli were present at the time of death.

II. SEROLOGICAL EXAMINATION OF THE BLOOD OF HEALTHY DOMESTIC ANIMALS FOR THE PRESENCE OF GAERTNER GROUP AGGLUTININS.

Systematic examination of the sera of domestic animals seemed a useful line of inquiry and one which might throw light upon the problem of Gaertner infections amongst these animals.

Apart from the horses the samples were all obtained from animals actually passed in public slaughter houses (Weston-super-Mare and London) as healthy and fit for human food. The method of examination was throughout the same, all agglutination determinations being microscopic with young broth cultures with a time interval of one hour for dilutions under 1 to 50 and one of two hours for all dilutions of 1 : 50 and higher. During this period the hanging drop preparations were kept at room temperature. Controls were of course made in the usual way. The horses were usually older animals most being 8—10 years old. For the most part they had been used for preparing diphtheria antitoxin and a few for other antitoxins. None had been given typhoid or Gaertner group inoculations.

The results obtained are shown in Table I.

TABLE I.

— signifies no reaction with a dilution of 1 to 20 in one hour.

Animal	No.	Approximate age	Positive agglutination limits		
			<i>B. enteritidis</i>	<i>B. suis-pestifer</i>	<i>B. paratyphosus B</i>
Calf	1	6-9 weeks	—	—	—
"	3	"	—	—	—
"	4	"	—	—	—
"	10	7-8 "	—	—	—
"	11	"	—	—	—
"	16	—	—	—	— *
"	24	—	—	—	—
"	25	—	—	—	—
"	26	—	—	—	—
"	27	—	—	—	—
"	40	3 weeks	—	—	—
"	55	"	—	—	—
"	56	1 month	—	—	—
"	117	4 weeks	—	—	—
Cow	42	3 years	200	20	20
"	49	1 year	20	20	50
Ox	37	3-3½ years	50	100	20
"	38	" "	50	50	20
"	39	3 "	200	100	20

* Para-Gaertner bacillus isolated from the spleen.

TABLE I—*continued*.

Animal	No.	Approximate age	Positive agglutination limits		
			<i>B. enteritidis</i>	<i>B. suis-pestifer</i>	<i>B. paratyphosus</i> B
Ox	41	3 years	50	50	50
"	43	3½ "	20	—	—
"	44	4 "	50	50	20
"	45	4 "	50	20	—
"	46	3 "	100	—	20*
"	47	3 "	50	20	20
"	48	4 "	100	50	50†
"	50	4 "	100	20	20
"	51	1½ "	100	20	20
"	52	4 "	100	50	50
"	57	3½-4 "	100	50	50
"	58	4 "	50	20	50
"	77	3¼ "	300	50	50
"	78	3 "	100	20	50
"	79	3½ "	100	20	50
"	80	2½ "	20	—	—
"	81	3 "	100	50	100
"	82	1¾ "	—	50	20
"	116	2 "	20	20	20
"	118	1½ "	100	100	100
"	119	2 "	50	20	50
Sheep	53	8 months	20	20	20
"	54	8 "	—	—	—
"	59	7-8 "	20	20	20
"	60	7-8 "	50	—	50
"	71	12 "	20	—	—
"	72	12 "	20	—	—
"	73	2 years	100	50	100
"	74	2 "	20	—	—
"	75	2 "	20	—	—
"	76	1½ "	20	—	20
"	114	1 year	20	—	—
"	115	1 "	50	50	50
"	120	1 "	—	—	—
"	121	1 "	—	—	—
"	122	1 "	100	—	—
"	123	1 "	20	—	—
"	124	1 "	20	—	—
"	125	1 "	—	—	—
Pig	5	—	250	20	100
"	6	—	100	—	50
"	7	—	100	100	100
"	8	—	100	—	—
"	9	6 months	—	—	—

* Abscess in liver: not bacteriologically examined.

† Actinomycosis; in retro-pharyngeal glands only.

*Bacilli of Gaertner Group*TABLE I—*continued*.

Animal	No.	Approximate age	Positive agglutination limits		
			<i>B. enteritidis</i>	<i>B. suis-pestifer</i>	<i>B. paratyphosus</i> B
Pig	12	4 months	20	20	20
	13	4 "	20	—	50
	14	4 "	20	—	20
	15	12 "	20	—	—
	17	—	—	—	—
	18	—	20	—	100
	19	—	100	—	50
	20	12 months	20	—	20
	21	12 "	100	—	100
	22	8 "	—	—	—
	23	8 "	20	—	—
	28	5 "	—	—	20
	29	5 "	—	—	—
	30	5 "	50	—	—
	31	5 "	20	—	20
	33	8 "	—	—	—
	34	8 "	250	20	50
	35	10 "	—	—	—
	36	10 "	250	—	20
	67	—	200	50	100
	68	—	100	20	20
	69	—	50	50	50
	70	—	50	20	50
	83	—	50	50	100
	84	—	50	20	20
	85	—	50	50	50
	86	—	50	20	20
	87	—	200	50	100
	88	—	200	20	50
	89	—	50	20	200
	90	—	—	—	—
Horse	61	—	—	—	—
	62	—	50	100	100
	63	—	100	100	20
	64	—	50	50	50
	65	—	—	—	—
	91	"aged"	—	20	20
	92	"	20	—	—
	93	"	20	—	20
	94	"	50	—	20
	95	"	20	—	—
	96	"	20	—	20
	97	"	20	—	20
	98	"	100	20	—
	99	"	100	50	50
	100	"	20	20	20

TABLE I—*continued*.

Animal	No.	Approximate age "aged"	Positive agglutination limits		
			<i>B. enteritidis</i>	<i>B. suispestifer</i>	<i>B. paratyphosus</i> B
Horse	102		100	50	20
"	103	"	50	—	—
"	104	"	20	—	—
"	105	"	—	—	—
"	106	"	100	20	50
"	107	"	20	—	20
"	108	"	—	—	—
"	109	"	50	20	50
"	110	"	50	20	20
"	111	"	—	—	—
"	112	"	50	—	20
"	113	"	50	50	50
"	126	"	50	—	50
"	127	"	100	20	20
"	128	"	—	50	50
"	129	"	100	50	50
"	130	—	—	—	—
"	131	—	50	50	20
"	132	—	50	100	100
"	133	—	—	—	50
"	134	—	50	50	50
"	135	—	50	20	20
"	136	—	20	—	—
"	137	—	100	50	50

My own investigations, and those of others in this country, show that while reacting sera may, and usually will, agglutinate differently with different members of the Gaertner group, they all agglutinate one or other of the three chief sub-group organisms and it is only necessary to test unknown sera with these three strains. *B. enteritidis*, *B. suispestifer*, and *B. paratyphosus* B. The actual strains used were for *B. enteritidis* the organism isolated by McWeeney from the Limerick outbreak, for *B. suispestifer* the strain I isolated from the Murrow outbreak or sometimes the strain isolated from the 1911 Chesterfield outbreak and for *B. paratyphosus* B a strain which I isolated from a para-typhoid fever case. The serological reactions and position in the group of all four strains had been very thoroughly worked out.

For convenience in considering the results they have been grouped as shown in Table II.

TABLE II.

Animal	No. examined	Sera classified into groups					Groups: percentages				
		A	B	C	D	E	A	B	C	D	E
Calf	14	14	0	0	0	0	100	—	—	—	—
Cow	2	0	0	1	0	1	—	—	50	—	50
Ox	24	0	3	8	11	2	0	12	33	47	8
Sheep	18	4	10	2	2	0	22	56	11	11	0
Pig	36	7	7	7	8	7	19	19	19	22	19
Horse	39	6	10	13	10	0	15	26	33	26	0
Totals	133	31	30	31	31	10	23.3	22.6	23.3	23.3	7.5

A = No trace of reaction.

B = trace of reaction, i.e. + in dilution of 1 : 20 with any one of the three organisms.

C = Slight reaction, i.e. + in dilution of 1 : 50 with any one of the three organisms.

D = Well marked reaction, i.e. + in dilution of 1 : 100 with any one of the three organisms

E = Very marked reaction, i.e. + in dilutions above 1 : 100 with any one of the three organisms.

If a positive reaction of 1 in 20 is disregarded as being of no significance and included with the negative reactions the following summary is obtained:

Animal	Percentages		
	No reaction (— and A)	Slight reaction (B and C)	Marked reaction (D and E)
Calf	100	—	—
Cow and ox	11	35	54
Sheep	78	11	11
Pig	38	19	41
Horse	41	33	26

Table I shows that the serum of a considerable proportion of the animals examined gave a positive reaction with Gaertner group strains. I only know of three possible explanations to account for the results.

(a) Due to the presence of "natural agglutinins" in the serum.

(b) Due to an old infection with Gaertner group organism and resulting in the formation of Gaertner group agglutinins in the blood.

(c) Due to infection with some unknown bacillus, or bacilli, not of the Gaertner group but sufficiently allied to this group to cause the serum to have some action on Gaertner group strains.

In the latter case the agglutination action would be of the nature of associated agglutinins. There is really no evidence in favour of this last hypothesis while it is inherently improbable. It cannot be entirely ruled out of consideration but is unlikely.

As regards the first hypothesis it is a recognized fact that perfectly normal serum may agglutinate certain bacteria to a limited extent but there is no satisfactory explanation as to the cause of the phenomenon. As far as I am aware "normal" agglutinins are not present in the new-

born animal and are therefore acquired during life. Such "normal" agglutinins are specific in the same way as ordinary agglutinins acquired as the result of infection and it is, therefore, a possible and not unreasonable supposition that their presence is due either to slight unrecognized infection with the specific bacilli or to the absorption of specific bacterial toxins from the intestinal canal.

In the present series the blood of all the 14 calves failed to agglutinate, even in a dilution of 1:20, any of the Gaertner strains, while 35 per cent. of the cows and oxen showed a slight reaction and 54 per cent. a marked reaction. It is a quite possible hypothesis that the older animals react because of Gaertner agglutinins produced either through toxic Gaertner products absorbed from the intestinal canal or because of slight infection of the animals with Gaertner bacilli, perhaps so slight in character that no appreciable symptoms were caused.

It would be of decided interest to take young calves and examine monthly their blood and note if the presence of these agglutinins coincided with the onset of slight illness or with the presence of Gaertner group bacilli in their excreta. Unfortunately material for this purpose has not been available to me.

The comparative absence of agglutinins in the sheep, an animal which extremely rarely causes food poisoning of Gaertner group origin, is a point of interest, but this may possibly be due to the fact that the sheep examined were not very old.

A number of the sera were also tested against *B. typhosus* and a certain proportion of them gave a positive agglutination reaction. In as much as the typhoid bacillus is not responsible for disease in animals and is never found in the animal intestine this might be taken as evidence of "natural" agglutination power and unassociated with any infection with pathogenic Gaertner or other group of bacilli. Two sets of observations however tend to show that this hypothesis cannot be maintained. The first is that in no case have I found that agglutination takes place with *B. typhosus* when failure to agglutinate the Gaertner strains was met with. The following are some sera which agglutinated *B. typhosus* in considerable dilution.

No.	Animal	Highest possible dilution with			
		<i>B. suipestifer</i>	<i>B. enteritidis</i>	<i>B. paratyphosus</i> B	<i>B. typhosus</i>
87	Pig	50	200	100	50
89	"	20	50	200	100
98	Horse	20	100	—	50
99	"	50	100	50	100
102	"	50	100	20	200
118	Ox	100	100	100	100 (200 partial)

In only a very few cases was the serum capable of agglutinating *B. typhosus* at a higher dilution than for the other bacilli used.

The matter was further tested by a few absorption tests. The results obtained are shown in the following Table.

TABLE III.

		Serum no. 99		Serum no. 118		Serum no. 89		Serum no. 102	
Bacillus		1:20	1:50	1:20	1:50	1:20	1:50	1:20	1:50
After absorption by <i>B. enteritidis</i>	Enteritidis	—	—	—	—	—	—	—	—
	Paratyphosus	—	—	—	—	+	+	—	—
	Typhosus	—	—	—	—	+	—	—	—
After absorption by <i>B. typhosus</i>	Enteritidis	+	+	+	+	+	+	—	—
	Paratyphosus	+	+	+	+	—	—	—	—
	Typhosus	—	—	—	—	—	—	—	—
After absorption by <i>B. paratyphosus</i> B	Enteritidis	not examined		+	+	—	—	not examined	
	Paratyphosus			—	—	—	—		
	Typhosus			+	+	+	—		

Note. The agglutination reactions of these sera before absorption are shown above.

The results are not absolutely uniform and this could hardly be anticipated in view of the very low titre of the sera used. They show no evidence of the presence of separate natural typhoid agglutinins.

In my paper upon "The Sources of Infection in Food poisoning Outbreaks" three papers are mentioned which may be mentioned here, as they throw definite light upon this question. In O'Brien's paper (1910) in a naturally occurring outbreak due to *B. suispestifer* (*aertrycke*) amongst laboratory guinea-pigs, of which nine survived the epidemic and were bacteriologically examined, five became carriers of the bacillus. The serum of four of these animals agglutinated this bacillus in dilutions of 1 : 50 and 1 : 100. As a control the sera of six stock guinea-pigs were tested and only one gave a reaction with a 1 in 20 dilution and none in higher dilution. The length of time during which these agglutinins persisted was not worked out. Petrie and O'Brien found that guinea-pigs fed with cultures may excrete the bacillus in the faeces for some time subsequently while remaining apparently healthy, and that the blood of some of these animals agglutinated the bacillus.

Reinhardt and Seibold fed a goat with massive doses of a Gaertner group strain. There was some slight rise of temperature but no definite illness. No agglutinins before feeding or four days after, but nine days after feeding the serum reacted in dilution of 1 : 40 and 16 days after the start of the experiment gave a positive reaction 1 in 2800. The animal was then killed. No pathological lesions were present and all the organs were sterile.

The available data is obviously insufficient to determine whether the positive reactions with Gaertner strains recorded above can be accepted as evidence of previous infection with these bacilli but they are suggestive of this.

Reaction of spleen pulp. Some continental workers have suggested that the serum reaction of the animal juices, particularly that of the muscles or spleen, might be of use as a rapid method for the diagnosis of infections from Gaertner group organisms.

In seven cases this possibility was tested by the examination of spleen pulp juice as well as the serum. All were from healthy pigs. Six of the sera samples gave positive agglutination reactions varying from 1 : 20 to 1 : 100 but in all seven cases no trace of agglutination was present when the filtered spleen pulp was used, even in dilutions of 1 : 20 and 1 : 10.

III. BACTERIOLOGICAL AND SEROLOGICAL EXAMINATION OF RATS.

In an investigation reported in 1913 Read and I studied a series of rats to ascertain how far they were infected with organisms of the Gaertner group. In all 41 rats were examined, the organs selected for examination being the liver, spleen, heart blood and intestinal contents. True Gaertner group organisms (all *B. enteritidis*) were isolated from five of them. All five were from Weston-super-Mare. Twelve days before two of these rats were examined, Danysz virus (a living virus which has been identified with *B. enteritidis*) had been distributed in different parts of the town, while in November, 1909, about 2½ years before the first rats were examined the refuse tips and slaughter houses had been extensively dosed with this virus. The positive rats were probably old cases of infection with this virus. Only a very few rat sera were examined but several agglutinated Gaertner strains to a considerable extent, i.e. 1 : 2000, 1 : 200, 1 : 500 in three cases.

In the present series this line of inquiry was followed up and further rats were examined.

This series comprised 48 rats all collected from Bristol or Avonmouth. The rats were being collected and examined by Professor Walker Hall for *B. pestis* in connection with plague infection in the city. They were all selected as rats which showed no obvious naked eye disease lesions and therefore serve well to study the extent to which Gaertner bacilli and agglutinins are present in apparently healthy animals. The spleen and heart blood were used for cultural examination and the latter also for

serological tests. These organs were removed and sent to me in sterile bottles. Cultural examination of the other organs was not considered necessary. The method of examination consisted of direct brushing from the interior of the organs on to a series of lactose bile salt neutral red agar plates.

Cultural findings. It is not necessary to give these in detail for each rat. In many cases the plates showed no growth but in a considerable number red colonies, presumably *B. coli*, were present. All white colonies were fully investigated. In a number of cases non-lactose fermenting white colonies were met with which failed to ferment glucose but as they fermented saccharose or salicin they were not further studied. In no instance was any true Gaertner organism isolated. On the other hand strains of para-Gaertner bacilli were isolated from five different rats.

These bacilli all possessed similar cultural characters. They were identical with true Gaertner bacilli in morphology, staining properties, characters of growth upon agar, gelatine and in broth, they fermented glucose but not lactose, saccharose or salicin. None produced indol, all five after a little acid production in litmus milk produced in 6—10 days marked alkalinity. Culturally they only differed from true Gaertner strains, as regards their action upon mannite and dulcitol. Four out of the five fermented mannite with gas production like true Gaertner strains but the gas produced was only slight in two. The fifth produced acid but no gas in mannite media. All five were sharply separated from true Gaertner strains by their failure to ferment dulcitol.

Their agglutination reactions also separated them, as none of the five were agglutinated, even in dilutions of 1 : 100, by *B. enteritidis*, *B. paratyphosus* B or *B. suispestifer* sera of fairly high titre (1 : 1000 to 1 : 4000).

The pathogenicity of only one strain was tested. The intraperitoneal inoculation into a young rabbit of as much as half an agar slope culture mixed with 1 c.c. of a 24 hours' broth culture failed to kill the animal or elicit any symptoms.

These organisms are interesting as culturally apart from the dulcitol test (except the one mannite acid producer) they are indistinguishable from the true Gaertner strains and where this test has not been employed have probably been taken for that organism.

Serological findings. In every case each serum was tested against the three organisms (*B. enteritidis*, *B. paratyphosus* B, *B. suispestifer*) of the group. The lowest dilution used was 1 : 50; all microscopic and two hours at room temperature.

Rather to my surprise no less than 45 of the 48 samples showed no reaction in 1 : 50 dilution with any of the above organisms. The only positive agglutination reactions were the following:

Rat laboratory no.	Limits of reaction			Source
	<i>B. enteritidis</i>	<i>B. suispestifer</i>	<i>B. paratyphosus B</i>	
522	—	—	100	Obtained from a ship.
378	50	50 (partial)	50	Bristol food shop.
380	50	50	50 (partial)	Slaughter house.

It will be seen that in no case had any marked agglutination power developed.

IV. BACTERIOLOGICAL EXAMINATIONS OF DOMESTIC ANIMALS SUFFERING FROM CERTAIN DISEASES.

As explained elsewhere there is a number of papers by continental investigators which demonstrate that infections with Gaertner group bacilli may occur amongst domestic animals. It is a singular fact that there are no reports, as far as I have been able to ascertain, of cases of this nature in Great Britain, apart from disease in animals associated with food poisoning outbreaks. The occurrence of food poisoning outbreaks in this country traced to diseased animals makes it probable that such occur and it is most desirable that careful bacteriological examinations into the cause of all deaths of this nature should be carried out. This would appear to be done but rarely. Unfortunately I have had great difficulty in obtaining such material and only the following have been examined.

No. 1. Pig found dead; cause of death not known. Putrefaction had set in when internal organ samples received. No Gaertner group bacilli could be isolated. The agglutination properties of the serum were not tested.

No. 2. Cow died from septic poisoning owing to rupture of womb during calving. A short chain streptococcus was isolated from the spleen. No Gaertner group bacilli could be isolated. The serum showed agglutinins, the limits of reaction being with *B. enteritidis* 1 : 500, *B. paratyphosus B* 1 : 100, *B. suispestifer* 1 : 50.

Evidently the animal did not die of a Gaertner infection. In view of the positive reactions with normal sera it is not possible to say if the agglutination reactions are evidence of old infection.

No. 3. For particulars of this case and for cultures from this case and No. 4 and No. 5 I am indebted to Sir Stuart Stockman. A ram ill with general cachexia for several days died and the post-mortem showed an acute catarrhal area in the urethra accompanied by cystitis and a chronic abscess in an inguinal gland. Bacteriological examination of the abscess showed a bacillus which on investigation was a variety of *B. coli*. No Gaertner organisms found.

No. 4. A lamb born dead practically at full time. The liver was discoloured and blood-stained oedematous fluid was present in both abdominal and thoracic cavities. The only organism which could be isolated was a typical strain of *B. coli*.

No. 5. Pig artificially infected with swine fever June 14th and slaughtered June 23rd. The liver and spleen were bacteriologically investigated but were not examined until over 24 hours after slaughter and removal. No true Gaertner group bacilli were isolated but it is of interest that two pseudo-Gaertner strains were isolated, both from the spleen. One of these differed culturally from true Gaertner organisms only in the fact that it failed to ferment dulse and definitely produced indol, while also it produced very little acid or gas in glucose media. The other differed only in that it failed to ferment dulcete, produced indol, fermented salicin and only gave slight alkalinity in milk. Both also were motile but much less so than true Gaertner strains. They were not agglutinated by the serum of this pig even in dilutions of 1 : 20.

The serum of this pig failed in dilutions of 1 : 50 and 1 : 100 to exert any agglutination power upon the three Gaertner group organisms.

No. 6. Serum and particulars of the case kindly sent me by Dr McGowan of Edinburgh.

The blood was obtained from the heart of a ten weeks old pig. It was one of a number of animals being killed out for swine fever. Post-mortem the animal showed extensive pneumonic consolidation of the middle lobe right side and upper lobe left side together with acute congestion of the rest of the lungs. Pleurisy was also present. The heart was healthy. Enlargement of the lymphatic glands generally. In abdomen nothing to note except acute inflammation of the small intestine, "so-called typical swine fever ulcers." No other inflammation. This serum showed no trace of agglutination in dilutions of 1 : 50 and 1 : 100 with the three Gaertner types or with *B. typhosus*.

SUMMARY AND CONCLUSIONS.

The examination of the spleens of 24 pigs and 10 calves and internal organs from 12 other pigs, all passed as healthy and fit for human consumption, failed to show the presence of any organisms belonging to the Gaertner group. One para-Gaertner bacillus was isolated in pure culture from the spleen of one of the calves.

The spleens of the 24 pigs and 10 calves were also examined for the presence of aerobic organisms generally. Exactly half of the pig and half of the calf spleens were sterile, the remaining 50 per cent. containing bacilli which were not completely identified but were mostly *B. coli*, non-lactose fermenters allied to *B. coli*, streptococci and staphylococci. All the organs were examined within a few hours of death. There was no correlation between interval since death and bacterial content nor any relationship to the age of the animal. The bacteria were evidently present in these organs at the time of death.

An extensive series of examinations of sera of animals passed as healthy showed that in a considerable proportion of them specific agglutinins were present against one or other member of the Gaertner group. These agglutinins were absent in all the calves, were mostly absent from sheep, but were fairly well developed in 40 to 50 per cent. of the pigs, cows and oxen examined. They were also present in a considerable proportion of the horses tested. The failure to demonstrate these agglutinins in the calf and their definite development in many of the cows and oxen sera suggest that they are not present in the new born animal but develop later in life. The available data is insufficient to enable a definite opinion to be given as to whether these positive reactions with Gaertner strains are to be ascribed to an old infection with these bacilli but they suggest this possibility.

As a practical point these results show that the demonstration of the presence of agglutinins, in moderate amount, against this group of bacilli in a suspected animal cannot be accepted in itself, and without further corroborative evidence, as proof of an existing infection with these organisms or as conclusive evidence connecting the animal with an outbreak of food poisoning. Much less so can the presence of such agglutinins be accepted as evidence warranting the condemnation of the carcase as unfit for food.

Amongst the animals examined suffering from definite disease one cow which died of septic poisoning showed the presence of a considerable amount of specific agglutinin in its serum, but the other bacteriological data was sufficient to say that the cause of death was not a Gaertner group infection.

The 48 rats examined were selected as showing no macroscopic evidence of Gaertner group infection and from none of them was a member of this group isolated, although five para-Gaertner strains were found which very closely resembled these bacteria.

In nearly every case the sera of these rats failed to show the presence of any Gaertner group agglutinins. This fact is of particular interest in view of the rather different findings which Read and I obtained with another series of rats from a different source and where the possibility of infection with Danysz virus (a Gaertner group organism) was likely. They add weight to the view that in these (mostly young) animals the presence of Gaertner group agglutinins to any considerable extent is evidence of an old infection with a member of this group, and is confirmatory evidence in favour of the agglutinin results with animals used for food being of a similar origin.

The absence of any correlation between the presence of the six para-Gaertner strains and the presence of agglutinins against true Gaertner bacilli is in favour of the view I have expressed from my earlier work, that there is no relationship between these two groups of organisms and that the para-strains cannot be considered as modified Gaertner organisms which under favourable conditions can revert to that type.

The examinations of specimens from diseased animals are too few to enable any deductions to be drawn, but I remain strongly of opinion that work along these lines is likely to throw valuable light upon the etiology of food poisoning and should be systematically undertaken by those who are in a position to obtain and examine such material.

IMPROVEMENTS IN THE TECHNIQUE OF THE CONCENTRATION OF ANTITOXIC SERA.

By ANNIE HOMER.

(From the Lister Institute, Elstree, Herts.)

THE study of the factors influencing the heat-denaturation of serum proteins has led us to change the technique of my method (1916) for concentrating antitoxic sera. These changes involve a considerable shortening of the process and therefore a brief description of our present method of procedure may be useful to those engaged in serum concentration in other laboratories.

Our technique is as follows:

(a) *The preliminary treatment of the plasma.*

In order to ensure the desired ease of filtration at the various stages of the process of concentration, the pooled batches of *undiluted* oxalated or citrated plasma are subjected to one of the following alternative methods of treatment previous to their being heated.

1. (a) To the plasma is added a sufficient volume of ammonia to adjust the reaction to the value $p_H + 8.0$. This may be accomplished as follows:

To 10 c.c. of the plasma is added the necessary volume (x c.c.) of a standard solution of ammonia to bring the hydrogen ion concentration $[H]$ to the value $p_H + 7.6$ using neutral-red as the indicator and Sørensen's solutions as standards. To another 10 c.c. of the plasma is added the necessary volume (y c.c.) of the same solution of ammonia to bring the $[H]$ to the value $p_H + 8.5$ (faint green tinge with three drops of a 0.1 per cent. solution of α naphtholphthalein).

By the addition of $\frac{x+y}{2}$ c.c. of the ammonia solution to every 10 c.c. of the plasma the reaction is approximately adjusted to the value $p_H + 8.0$.

(b) If the operator cannot conveniently adjust the reaction, then to the plasma can be added not less than 0.25 and not more than 0.30 per cent. of cresylic acid or trikresol.

2. Two per cent. of solid sodium chloride is next added to the plasma treated as above.

(b) *The heating of the plasma.*

The heating is conducted in two stages as follows:

Stage 1. Into each of several jars of 22 litre capacity there are measured 14 litres of plasma. The jars are covered and placed in the heating tank containing water at 62°—64° C. whose temperature is maintained between these limits until that of the plasma has reached 57.5° C. The water in the tank is then allowed to cool to 57.5°—58° C., at which temperature it is maintained by means of a thermostat. The tank is covered with a closely fitting lid and *the heating is continued for a further period of four hours.*

Stage 2. At the expiration of the stated period for Stage 1, six litres of a saturated solution of ammonium sulphate are added to each of the jars. The ammonium sulphate content in each jar is thus brought up to 30 per cent. of saturation.

The temperature of the water in the tank is raised to 62°—63° C. and is kept between these limits until the temperature of the plasma-ammonium sulphate mixtures has reached 58° C. Throughout this operation it is essential to keep the mixtures thoroughly well stirred.

As soon as the required temperature has been reached, the jars are removed from the tank and the contents are allowed to cool to 45° C. at which temperature they are filtered. The precipitate thus separated constitutes the First Fraction precipitate.

(c) *The precipitation of the Second Fraction.*

The First Fraction precipitates, consisting of euglobulin, heat denaturated protein and a certain amount of pseudoglobulin, are washed with a solution of ammonium sulphate (30 per cent. of saturation), the volume of the latter being about one-half that of the plasma taken for concentration.

The washings from the First Fraction precipitates are filtered and added to the main bulk of the filtrates to which is then added the necessary volume of a saturated solution of ammonium sulphate *to bring the ammonium sulphate content up to 46 per cent. of saturation.*

The ensuing precipitate is filtered, pressed and dialysed in the usual way until free from sulphate. To the residue from dialysis is added 0.85 per cent. of sodium chloride and 0.35 per cent. of cresylic acid. This final product is stored in bulk in the cold room until required.

(d) *The recovery of antitoxin from the First Fraction Precipitates.*

During many years practical experience with the concentration of antitoxic sera in these laboratories it has been found that, no matter how carefully any given method be followed, there has always been a loss of antitoxin, the losses under ordinary circumstances ranging from 5 to 30 per cent. (average *ca.* 18 per cent.).

It has been ascertained that the "missing" antitoxin is not destroyed during the heating of the serum and of the serum mixtures at the temperatures employed, but that it is carried down with heat-denaturated pseudoglobulin in the First Fraction precipitates. The antitoxin is associated with the denaturated protein in such a manner that it cannot be recovered therefrom by extraction with ammonium sulphate (30 per cent. of saturation). However, it has been found that this "missing" antitoxin can be almost completely recovered by prolonged extraction of the First Fraction precipitates with brine in which the heat denaturated protein is soluble to some extent.

In order to reduce our losses to a minimum we have therefore reverted to the Banzhaf-Gibson plan of extracting the First Fraction precipitates with brine, which was long employed in this laboratory. At the same time the method was adopted of dealing with the First Fraction precipitates from a series of concentrations collectively and, since Dr MacConkey¹ had demonstrated that antitoxic sera saturated with salt could be kept at room temperature for months without any appreciable deterioration, his plan of allowing them to soak in brine until they could be conveniently treated, was employed, labour and expense being thereby saved.

Our procedure is as follows:

The First Fraction precipitates after having been washed with a solution of ammonium sulphate (30 per cent. of saturation), are placed in a coarse canvas bag. The bags are dumped into tubs containing a saturated solution of common salt with an excess of solid salt.

Into the same tub are successively dumped the similar precipitates from a series of weekly routine concentrations, the First Fraction precipitates from the concentration of 500 to 600 litres of plasma being

¹ Unpublished observations.

usually thrown into about 180 litres of brine. The tubs are kept covered and allowed to stand at room temperature until time can be spared to deal with the liquids. The brined extract of the precipitates is then filtered and to the filtrate is added 0.30 per cent. of glacial acetic acid. The ensuing precipitate is filtered, pressed and dialysed after 3 per cent. of finely ground washing soda has been added to the contents of each parchment dialysing bag. Care must be taken not to overpress the precipitates as in this eventuality great difficulty is experienced in dealing with the residues from dialysis.

The dialysis is continued until the contents of the dialysing bags are freed from salt. To the residues from dialysis are added 0.85 per cent. of solid sodium chloride and 0.35 per cent. of cresylic acid. The final product is stored in the bulk in the cold room until required.

The above technique presents the following practical advantages over that originally suggested by me (1916):

(1) The preliminary adjustment of the plasma obviates difficulties otherwise so often experienced in the filtration of the hot plasma ammonium sulphate mixtures and also of the final products.

(2) The shortening of the heating in Stage 1 makes it possible to complete the two stages of the heating process within 7 hours.

This is advantageous from the practical standpoint. In a working day of 8 hours' length, the operator can not only carry out the heating processes involved but can also leave the filtration of the First Fraction proceeding overnight.

(3) The precipitation of the Second Fraction by 46 instead of 50 per cent. of saturation with ammonium sulphate considerably reduces the amount of heat-denaturated albumin appearing in the final product. This is advantageous in two ways, for, not only is the degree of concentration enhanced but the colour of the final products is less pronounced.

(4) At a relatively small cost of labour, time and materials the total losses of antitoxin experienced during the concentration of sera can be reduced to a negligible quantity.

Thus, during a series of routine concentrations involving nearly 1500 litres of plasma, the loss with each individual concentration was 15—20 per cent. The subsequent treatment of the First Fraction precipitates with brine resulted in the recovery of an amount of antitoxin which reduced the total loss to 2.5 per cent. These results, based on animal tests, indicate that there had been practically no loss of antitoxin during the process of concentration.

Our present losses are considerably smaller than those experienced with the Banzhaf-Gibson method in which, in order to ensure clear and readily filterable end products, it has been found necessary to throw down the First Fraction precipitates with 33 or even with 36 per cent. of saturation with ammonium sulphate instead of with 30 per cent. as originally advocated.

Recent experiment has shown that, with the higher concentrations of ammonium sulphate thus adopted, the precipitated heat denaturated pseudoglobulin and attendant antitoxin are so influenced that their solubility in brine is considerably decreased. Under these conditions extractions of the First Fraction precipitates with brine does not reduce the loss to the minimum experienced by us in our present procedure.

The similar treatment of the First Fraction precipitates obtained in the Banzhaf (1913) method and in my modification of the latter likewise reduces the loss to a minimum.

In conclusion I desire to express my thanks to Mr Albert Riggs, Head Laboratory Assistant, for his co-operation and for the many valuable suggestions made by him during the working out of the details of the technique described above.

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THE HISTOLOGICAL CHANGES IN FROZEN FISH AND THE ALTERATIONS IN THE TASTE AND PHYSIOLOGICAL PROPERTIES OF THEIR FLESH.

A RESUMÉ OF DR K. REUTER'S INVESTIGATIONS¹.

BY G. H. F. NUTTALL AND J. STANLEY GARDINER.

(With Plate I.)

[Up to recent years, fish were frozen in a dry chamber; this was not found entirely satisfactory, the fish tending to become dry and lose their flavour. We were ourselves intending to experiment on freezing fish in brine prepared in different ways and made a proposal to the Fish Food and Motor Loan Committee to this effect. Our attention was then drawn to Ottesen's process which we have since tested through the kindness of Mr J. V. Pryor of Cambridge², who has supplied us with ice and allowed us to use his cold storage chambers. The confirmatory results, which we have obtained practically, are well explained in the light of Dr Reuter's report on his careful investigations, an abstract of which follows.

The preservation of fish and other food is clearly a matter of great national importance, and it already exercises the attention of the Governments of all the Allies. We believe, therefore, that the results of the researches herein summarized should become widely known.]

The expansion of water on freezing formerly dominated our views as to its effects on the dead animal body. The idea arose that the body fluids on freezing rupture the tissues so as to burst such organs as the

¹ "Ueber die histologischen und geschmacksphysiologischen Veränderungen gefrorener Fische," von Dr med. Karl Reuter, Hamburg. *Abhandlungen zur Volksernährung*, Berlin, 1916, Heft 5, pp. 211-242, figs. 36-55.

A copy of the foregoing publication was obtained recently through the good offices of the Fisheries Branch of the Board of Agriculture and Fisheries. We reproduce nine of the author's illustrations.

² "Frozen Fish." *Fish Trades Gazette*, Jan. 19, 1918, p. 23.

intestine, the urinary and gall bladders. The tissues on thawing were supposed to be broken up and putrefaction to result, since putrefactive changes became more apparent after freezing. In fish it was thought that the rupture of the gall bladder would especially lead to a deterioration in the flesh. However, no such bursting actually occurs, the walls of such hollow organs possessing sufficient elasticity to resist the expansion due to freezing.

The fine histological changes which tissues undergo through freezing are of greater significance. They chiefly affect the blood and muscle. The haemoglobin of the former becomes dissolved in the serum (haemolysis). It then diffuses into the muscles staining them faintly red; a similar change also occurs as a result of putrefaction. Such diffusion in fish is small, discolouration being seen only near the vertebral column in the neighbourhood of the large caudal artery and vein; elsewhere the muscles showing the grey-white colour characteristic of fish muscle.

Fish, whether frozen rapidly in brine or slowly in air, present at first a similar external appearance. The bright glossiness due to the slime is, however, quickly lost in the air-frozen fish, the skin of which soon commences to present a shrivelled appearance somewhat approaching that seen in dried fish. In such fish loss of weight may be considerable, complete drying being ultimately effected if the frozen fish is kept in air for any length of time.

Preliminary examinations were made of the muscles of various fish immediately on killing. Small pieces were cut out and frozen by liquid CO_2 . They were then cut into sections of 0.01 mm. thickness by a freezing microtome. Such sections show the principal changes in the muscles due to freezing and are best understood by reference to schematic Figs. 1 and 2 (Pl. I) of unfrozen and frozen muscles. In Fig. 1 the muscle fibres appear as fine prismatic columns, consisting of the sarcolemma enclosing the sarcoplasm, or contractile substance. In rapidly frozen muscle (Fig. 2), the contents of the muscle columns have become separated into central more fluid and peripheral less fluid parts. The latter are pressed against the sarcolemma, and the fibres simulate hollow cylinders enclosing ice columns. While the whole muscle is frozen hard, histological changes only occur in the fibres, not in the connective tissue binding them together.

In fact, freezing separates the water from the albumin in each muscle fibre so that it comes to lie axially. Such a separation clearly can only take place during the freezing process, for with complete hardening there can be no further histological changes. Most fish begin to freeze

at about -1°C. , and at this temperature by far the greater amount of fluid contained in the fibres separates out. It may be assumed that the water which collects in the centre of the fibre contains salts. Furthermore, sections treated with alcohol exhibit a finely granular amorphous precipitate of albumin in this fluid, which it is suggested has about the same chemical composition as the juice which escapes on thawing.

Numerous histological examinations of fish muscle had necessarily to be made in the course of the investigations. Killing in 4 % formalin, hardening in graded alcohols and embedding in celloidin gave the best results. The shrinkage was slight and large sections were cut, at times extending through the whole body of the fish (Pl. I, Figs. 6 and 7). The study of these sections gives rise to three questions:

(1) Can the change in the tissues be altered by the method of freezing? Is rapid or slow freezing the better?

(2) Can the process of thawing be modified so as to induce the tissues to return to their original state?

(3) How far is the value of fish as food changed by freezing? Keeping qualities, nutritive value and flavour?

In the first experiments, made both with fish rapidly frozen at a low temperature in brine and with fish slowly frozen at a moderate temperature in the air of a refrigerating chamber, a difference was visible to the naked eye on cutting the muscles. In the slowly frozen fish relatively large crystals of ice formed between the muscular fibres giving rise to clear spaces after thawing, the flesh appearing translucent. In rapidly frozen fish no structural changes were thus visible, the flesh appearing uniformly opaque. After thawing these differences became less marked, because, as the tissues softened, the interspaces disappeared. Under otherwise similar conditions more of the juice drained off the slowly frozen than off the rapidly frozen fish.

The microscopic appearances differed considerably. Where the freezing was most rapid, viz. close under the skin, a number of small ice columns were formed within the muscle fibres (Pl. I, Fig. 3). In deeper muscular fibres, where freezing was less rapid, the number of such columns decreased so that finally each fibre contained a single column. In large fish, in the centre of the musculature the escape of fluid out of the fibres was observable, this being due to the rupture of the sarcolemma (Pl. I, Fig. 4).

In fish frozen slowly in the air vacant spaces corresponding to the ice columns no longer occur in the sarcoplasm. The muscle fibres form compressed bands or separate bunches of sharp-edged little columns,

while irregular spaces appear in the connecting tissue into which the fluid from the muscle fibres has exuded. In other words the fluid congeals outside the muscle fibres (Pl. I, Fig. 5).

The explanation of these differences is to be found in the colloidal state of the muscle. It has been seen that water is separated from the gelatinous content of the muscle fibre. In all colloids the time required for imbibition, and the reversal of this process, is much longer than in bodies having a crystalloid structure. The assumption is therefore justified that the separation of water from a colloid will be more complete the more slowly it is cooled down to the point at which it solidifies. Conversely the separation of fluid will be lessened in proportion to the rapidity with which the temperature of solidification is attained. By using liquid CO_2 small pieces of muscle could be so rapidly frozen that no changes were produced. Freezing took place within one or two seconds, and sections only showed the effect of freezing on their edges, the greater part of the tissue presenting the appearance of normal unfrozen muscle (Pl. I, Fig. 8). No exudation of fluid into the connective tissue, and no aggregation into columns within the separate fibres could be seen. The muscle illustrated in Pl. I, Fig. 9, the freezing of which lasted five to ten minutes, may be compared to that in Fig. 8, the latter being practically the section of a normal unfrozen muscle and comparable to schematic Fig. 1, and Fig. 9 corresponding to schematic Fig. 2.

The conclusion from the above observations is that in very rapid freezing the water of the muscle albumin freezes in an invisible molecular state. In less rapid freezing a number of small columns of fluid are formed in each muscle fibre, if time allows, these fusing to form a single column. In still slower freezing the fluid ruptures the sarcolemma and escapes into the connective tissue, forming large spaces filled with ice.

In brine-frozen fish the spaces between the muscle fibres are not visible to the naked eye, but in air-frozen fish ice crystals may be seen, although at times the fibres may remain intact. Indeed it is evident that in different methods of freezing all gradations may be found. The size and character of any fish, its possession of a thick skin, an isolated layer of fat, a swimming bladder, or a peculiar shape may influence the changes brought about by freezing and explain occasional small divergences. The essential difference observable in a fish frozen in the air of the refrigerating room at -7° to -12°C . and in one frozen in brine at -15°C . is always attributable to the difference in sizes of the ice crystals formed. It explains why on thawing less juice escapes from

the flesh of brine-frozen fish, its small lacunae possessing greater capillary attraction. The juice, however, can by slight pressure be squeezed out, and this is a drawback to frozen fish, readily differentiating it from the unfrozen¹. It is of practical importance that such thawed fish should not be exposed to pressure and moreover desirable that it should in preparation for table be cut as little as possible.

When slow thawing occurs in the presence of a great deal of juice, the cells being ruptured, the muscle fibres only act as passive bodies in the fluid. Experiments show that the muscle albumin does not reabsorb its fluid, unless the duration of the frozen condition has been very short. In the latter case there is a slight recovery after thawing, but muscle albumin does not give off and reabsorb water appreciably as do such colloids as glue and gelatin.

The best results in thawing were obtained by floating the fish in a large vessel of cold water. According to our investigations the speed of thawing has no influence on the recovery of the tissues from the effects of freezing. These observations are not in agreement with those of the Dutch Report wherein the authors considered that they had observed such a recovery. As the measure of the changes may be subject to the personal equation, a series of measurements are now being made. The author's observations convince him that recovery is very improbable and of no practical importance. The fact that the muscle albumin does not reabsorb the expelled fluid indicates that its colloidal condition has undergone changes closely similar to those of coagulation. In this respect Ostwald states of colloids that when they freeze "*in der Regel gleichzeitig mit der Kristallisation des Eises das Kolloid koaguliert, obschon in vielen Fällen die Homogenität der räumlichen Verteilung annähernd gewahrt bleibt,*" a statement which does not always apply to muscle. The muscles of cod were compared frozen both in brine and in the air, allowing them to thaw at room temperature. Coagulation seemed to increase with the length of time that the fish remained frozen. If the freezing has only been maintained for a short time, the muscle retains some of its viscosity and elastic-gelatinous consistency; if frozen for a long time, it loses its elasticity and becomes dry and friable. The difference is one which can easily be determined if pieces of the muscle are pressed between the fingers.

¹ [Our experience with brine-frozen herring does not agree with Dr Reuter's statement which apparently applies to cod; the juice cannot be easily expressed in this manner, and differentiation between frozen and unfrozen herrings is exceedingly difficult. G. H. F. N. and J. S. G.]

The nutritive value of frozen fish may be reduced owing to the loss of meat juice, but on the other hand freezing is an advantage in that it prevents putrefaction. The digestibility of fish muscle may possibly be increased by the loosening of the fibres by freezing. Experiments were made by digesting with artificial gastric juice at body temperature. Fresh, slowly and quickly frozen fish were compared, but no differences were found. The flesh of slowly frozen fish is somewhat firmer than that of fresh fish, the muscle fibres being pressed together and of a straw-like consistency. The differences in quickly frozen fish are so small that they can neither in texture nor in taste be readily distinguished from fresh fish.

The natural smell of fish depends on volatile substances and may be of considerable value in rendering them appetising. These substances are absorbed by the brine or given off into the air, being doubtless present mainly near the surface of the fish. To such a degree is this the case that brine before being used for further freezing has to be filtered through charcoal. All haemoglobin is converted into oxy-haemoglobin so that the blood appears bright red in frozen fish instead of dark coloured as in ordinary dead fish.

The progressive drying of frozen fish during storage, accompanied as it is by gaseous interchange (oxidation) and loss of aroma, may make a considerable difference to the flavour, leading to a tastelessness, which is also a frequent source of complaint against frozen meat. It is probably owing to this oxidation that fish, which contains a small quantity of fat such as mackerel and herring, may acquire a marked rancid flavour. To prevent this care must be taken to exclude air either by covering the stored fish by an impermeable membrane or by other means.

Finally, Dr Reuter desires to make it clear that his conclusions only apply to fresh fish. If putrefaction has begun prior to freezing, other changes may take place in the fish during freezing and storage: his experiments do not cover such fish. He assumes that all bacterial and enzyme action ceases in frozen fish as otherwise meat could not be preserved by cold storage. The bacteria and enzymes, however, return to activity on thawing. Moulds, moreover, may grow on frozen fish or meat if the temperature in the storage chamber rises above a certain point, air being allowed free access.

EXPLANATION OF PLATE I.

Figs. 1-5 are schematic. For description, see the text.

- Fig. 1. Unfrozen muscle (corresponds to Fig. 8).
- Fig. 2. Frozen muscle (corresponds to Fig. 9).
- Fig. 3. Rapidly frozen muscle (brine process, at -14° to -16° C.).
- Fig. 4. Less rapidly frozen muscle (deep-seated muscle of fish treated as under Fig. 3).
- Fig. 5. Slowly frozen muscle; the connective tissue not represented (fish exposed to air in chamber at -7° to -12° C.).

Figs. 6-7. Photographs of cross sections of whole fish (reduced in size).

- Fig. 6. Brine-frozen cod. But few interspaces are visible between the muscles; the interspaces are partly attributable to displacement in cutting the flesh and partly to shrinkage in alcohol.
- Fig. 7. Slowly air-frozen cod. Numerous large interspaces arranged symmetrically are visible between the muscle bundles, the grouping of the latter is due to the distribution of connective tissue amongst the bundles. There is considerable shrinkage.

Figs. 8-9. Photomicrographs of cross sections of fish muscle.

- Fig. 8. Very thin section of fresh cod's muscle frozen by liquid CO_2 in a few seconds. Photographed whilst in salt solution and unstained, $\times 180$ (corresponds to schematic Fig. 1 and shows the appearance of normal muscle).
- Fig. 9. Somewhat thicker section than that shown in Fig. 8. Fresh plaice's muscle frozen by liquid CO_2 in 5-10 minutes. Photographed in salt solution and unstained, $\times 135$ (corresponds to schematic Fig. 2).

LETTERING TO FIGURES.

f, muscle fibre; *i*, connective tissue; *k*, nuclei of muscle fibre; *l*, large lacunae due to expansion from ice; *p*, sarcoplasm; *R*, small intramuscular spaces (see description of Fig. 6); *s*, sarcolemma; *Sr*, shrinkage leading to folds in skin; *w*, ice columns; in Fig. 9 they represent spaces previously occupied by columns of ice as shown in schematic Fig. 2.

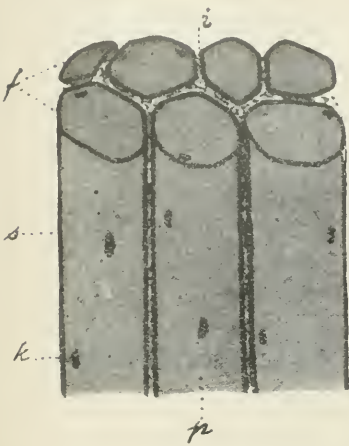


Fig. 1

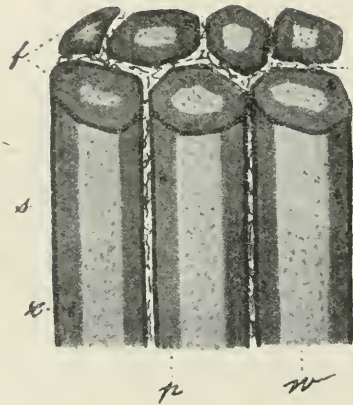


Fig. 2

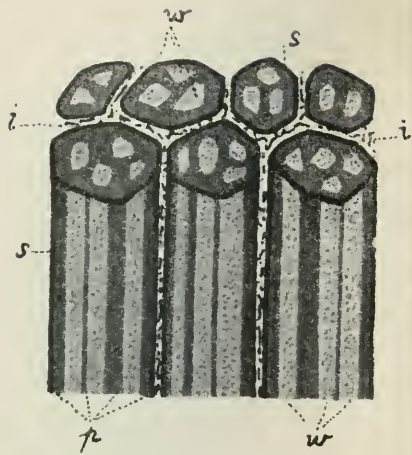


Fig. 3

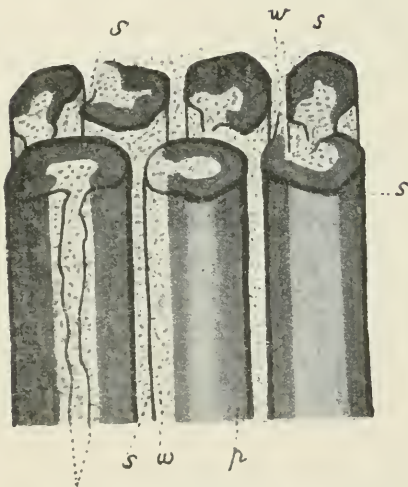


Fig. 4

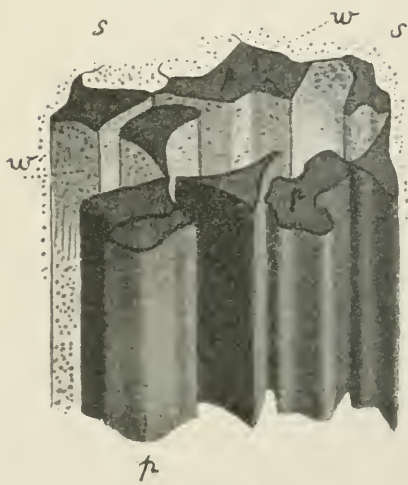


Fig. 5

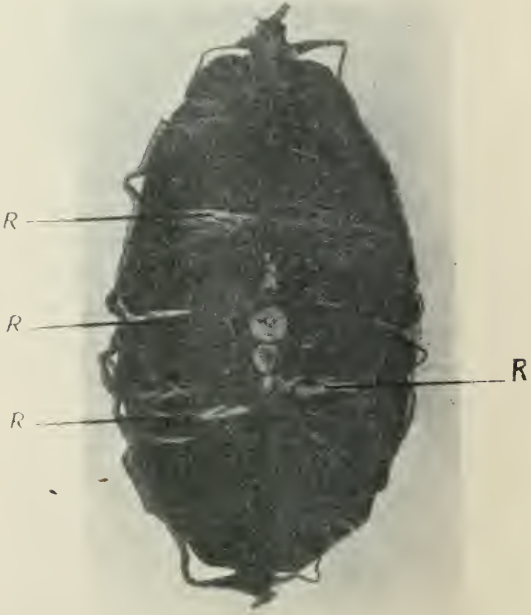


Fig. 6

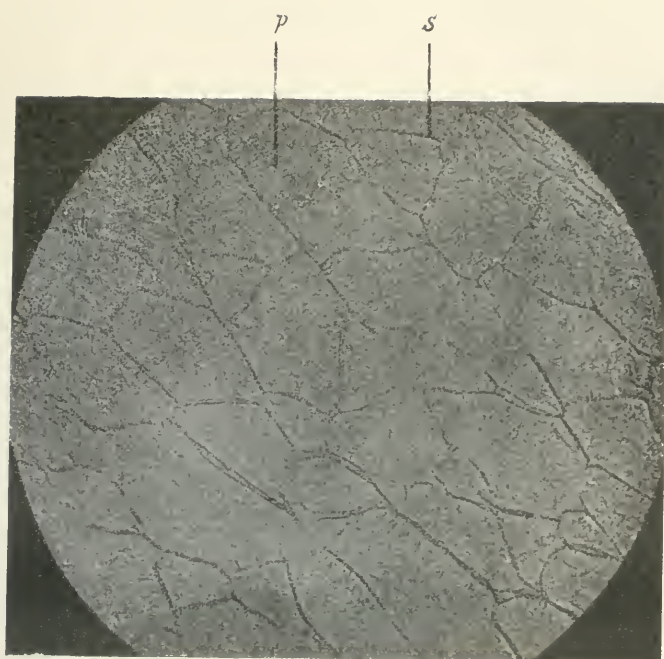


Fig. 8

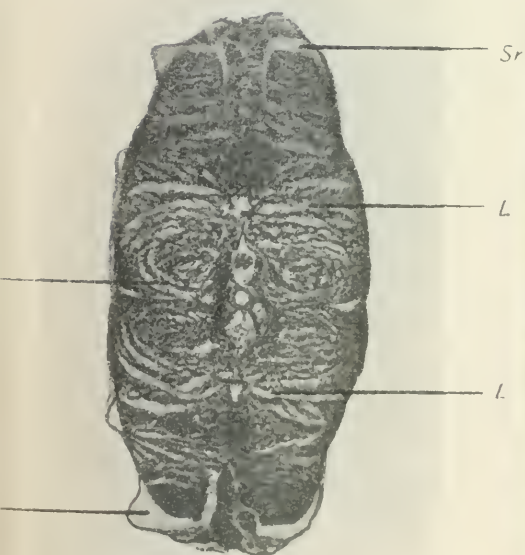


Fig. 7

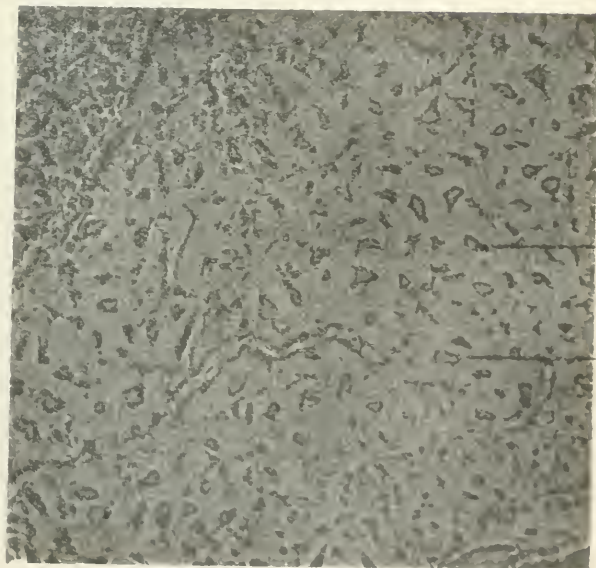


Fig. 9

SECOND REPORT ON BACTERIOLOGICAL ASPECTS OF THE MENINGOCOCCUS CARRIER PROBLEM¹.

By ARTHUR EASTWOOD, M.D.

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¹ Reprinted from *Reports to the Local Government Board on Public Health and Medical Subjects*, n.s. No. 114 (1917), by permission of His Majesty's Stationery Office.

INTRODUCTION.

IN my first report¹ I showed that in the throats of persons not known to have been in contact with cases of cerebro-spinal fever organisms were frequently found which were indistinguishable from meningococci isolated from the cerebro-spinal fluid of persons suffering from that disease.

During 1916 the investigators in the Board's Laboratory have continued to make further bacteriological inquiry into the characteristics of such naso-pharyngeal organisms, and into their relationship to strains obtained from the cerebro-spinal fluid in cases of meningococcal meningitis. In addition to Drs F. Griffith and W. M. Scott, who have been occupied with this research throughout the year, Captain C. W. Ponder, R.A.M.C., has taken part in the investigation since June, 1916, when he was loaned to the Board by the War Office for this purpose.

Before discussing scientific details it will be useful to set out the main objects of the present work in general terms.

With the view of ascertaining whether further investigation would corroborate the previous year's findings, more naso-pharyngeal swabs from non-contacts have been examined, and this inquiry has been extended into localities distant from those previously investigated. The results have been confirmatory of the previous work, in that many additional non-contact strains have been found which are indistinguishable from meningococci of cerebro-spinal origin.

Whilst these facts point to a wide distribution of the meningococcus amongst the population of the areas investigated, it is necessary, before drawing general conclusions, to consider whether the tests applied for the identification of this organism have been sufficient, and whether some test might not be found which would serve to differentiate meningococci of cerebro-spinal origin from the majority, at least, of those which occur in the throats of non-contacts. With this object Drs Griffith and Scott and Captain Ponder have paid special attention to serological reactions, as possibly affording a means of differentiation.

The problem would be simple if it were possible to adopt one meningococcal serum as the standard and to lay down the law that naso-pharyngeal strains which are agglutinated by this serum are meningococci, and that strains which are not agglutinated by it are not meningococci. But any such simple solution is quite out of the question. It is clear from the last reports by the Board's investigators that undoubted

¹ *Journ. of Hygiene*, xv. 405.

meningococci of cerebro-spinal origin differ among themselves in their serological reactions, and that no one serum is available which will agglutinate them all. This fact has been amply confirmed by other bacteriologists.

Can the difficulty be met by using a larger number of standard meningococcal sera, on the hypothesis that, if each variety of true meningococcus be represented serologically, an organism which agglutinated with at least one of the sera would be a meningococcus, whilst failure to agglutinate with at least one serum would exclude an organism from this class?

This hypothesis raises important issues which must first be clearly defined, and then be examined in the light of laboratory data.

(1) How many standard sera would be required? If two or three would suffice, it would not be impracticable to test each unknown strain against each serum; but if a large, and perhaps indefinitely large, number of sera would be needed, the method would be impracticable for routine diagnosis.

(2) Would it be possible to establish identity of standards? One laboratory might adopt a certain set of sera as being the most useful for differentiation, and another laboratory, with equal right of scientific authority, might adopt a different set, which might give different results. Then the decision as to what was or was not a meningococcus would be no more than the expression of a personal opinion, and would vary according to the views of the particular investigator.

(3) Would the results of simple agglutination tests necessarily be diagnostic? One knows that for certain organisms—*e.g.* the typhoid bacillus and the cholera vibrio—the ordinary agglutination test is extremely useful, and, indeed, invaluable; and though the bacteriologist has to keep on the alert for possible fallacies, the need for caution does not detract from the fact that, with these organisms, the agglutination test is of very great practical utility. But agglutination with a coccus is much more irregular, and so many precautions have to be taken to avoid error that the question arises, certainly in the case of the meningococcus, whether the result of a simple agglutination test can be accepted as a final criterion for routine diagnosis.

(4) Is there any way of improving the agglutination test so as to overcome the difficulties met with in the meningococcus? The difficulties are twofold, and are similar to the difficulties encountered in serological tests with other organisms. Sometimes (*a*) a standard meningococcus serum may agglutinate organisms which are not meningococci—*e.g.* it

may agglutinate gonococci; and sometimes (*b*) an undoubted meningococcus may fail to agglutinate with any standard serum, just as a pneumococcus may fail to agglutinate with any standard pneumococcal serum, although it may be known to have produced lobar pneumonia. A suggested way out of the difficulty (*a*) is based on the principle that if a serum prepared from an organism A agglutinates not only A but also a different organism B, these two different capacities of the serum can be separated out by treating the serum with a culture of B and then removing the deposit; the clear liquid remaining will be found to have lost its power of agglutinating B but to have retained its power of agglutinating A. If the serum had been treated with a culture of A, instead of B, and then retested, its power of agglutinating A would have disappeared. This indicates a method of ascertaining whether an unknown organism which is agglutinated by serum A (a meningococcal serum) is identical with A (a meningococcus) or is really some different organism (B). It also suggests (*b*) a method of ascertaining whether a culture of an unknown organism which is not agglutinated by serum A is of the same type as culture A; if it is, the serum, after treatment with the culture in question, will have lost its power of agglutinating culture A; but if it is not, the capacity of the serum for agglutinating culture A will not be affected. These considerations naturally raise the question whether the proposed method of differentiation by "the absorption of agglutinin test" is reliable for the identification or differentiation of meningococci.

(5) The question last raised involves general principles of bacteriological classification, and the answer to it must involve consideration of these. The definite issue raised is whether the principle of differentiation by capacity for absorption of agglutinin is valid for the classification of organisms which, in other bacteriological respects, are essentially indistinguishable from each other. The practical importance of this problem may be illustrated by a well-known example. In certain intestinal disorders and in suspected food-poisoning there are two organisms, amongst others, which it is important to identify, viz. *B. paratyphosus* (B) and a widely-distributed organism known as *B. suispestifer*. These two are closely allied in their bacteriological reactions, and a good deal of research has been devoted to the question of their inter-relationship, both in England and in Germany. Opinion is still divided between two opposite schools, the one holding that the two organisms are clearly distinguishable by the absorption method, whilst the other maintains that both belong to the same group, and that they cannot be differentiated

either by absorption or by any other bacteriological means. This illustration is particularly apposite to the question whether meningococci can be divided into (a) a pathogenic class and (b) a common but relatively harmless saprophytic class, because in the meningococcal problem the questions of scientific methods of differentiation and classification are identical with those raised in the controversy regarding *paratyphosus* and *suipestifer*.

(6) So far, I have only referred to the diagnosis of non-contact strains by means of sera prepared from cerebro-spinal meningococci. What further evidence of inter-relationship or differences would be obtained if sera were prepared from naso-pharyngeal strains and were fully investigated? Such information is obviously required, though the preparation of sera from unknown strains would not be practicable as a part of routine diagnosis.

(7) All these questions mean that serological diagnosis may be inaccurate unless it is based upon a correct appreciation of the limitations of the method employed.

It is therefore necessary to examine current assumptions about the principles of immunity, in so far as they affect the interpretation of certain serological reactions, and to enquire into the validity of their application to the differentiation of species.

(8) After due consideration of the above questions the final issue as to the diagnostic value of serological tests may be brought to a focus. The crucial question will be—a naso-pharyngeal strain is tested serologically and is found to give reactions which do not identify it as a meningococcus though it is identical with meningococci morphologically, culturally, and in fermentation tests. Is such an organism to be regarded as possibly capable of producing cerebro-spinal fever? And if the answer is in the negative, the nature of the required serological identity must be defined.

THE DISTRIBUTION OF THE MENINGOCOCCUS AMONGST THE GENERAL POPULATION.

THE USE OF THE TERM "NON-CONTACT."

With reference to the work of the Board's investigators on the carrier problem it will be useful to clear up certain ambiguities attaching to the significance of the convenient terms "contact" and "non-contact."

There is, I believe, general agreement on the following matters:—

(1) Cerebro-spinal fever develops in persons who, prior to the onset

of the disease, have "carried" the meningococcus in their nasopharynges.

(2) The number of persons who develop the disease is very small in proportion to the number of carriers.

(3) The meningococcus is disseminated amongst the population by contact with carriers.

(4) When a person develops cerebro-spinal fever, some of the persons who have been in intimate contact with him will also, in all probability, be found to be carriers. Such carriers may be termed, collectively, Group I.

(5) It cannot be assumed that each member of Group I became a carrier owing to contact with the person who developed the disease; it is quite possible that the patient derived his infection from one of these healthy carriers.

(6) Persons not in contact with the patient may have been in contact with one or other of the persons A, B, C, etc., who constitute Group I, and may have become carriers in consequence, thus forming Groups A, B, C, etc.

(7) Similarly each individual in Groups A, B, C, etc. may be the focus of another group; and so the process may go on indefinitely.

(8) Carriers may retain the meningococcus in their throats for a long time, though not, as a rule, for more than two or three weeks.

It is thus evident that a case of cerebro-spinal fever can usually be regarded as associated, directly and indirectly, with an indefinitely large number of carriers, of whom (a) some are known to have been in contact with the patient; (b) a larger number can be found on enquiry to have been associated, directly or intermediately, with (a); and (c) a still larger number are intermediately connected with (a), but the connecting links cannot be traced. Then the rest of the population would comprise (d) all the persons, whether carriers or not, who have no connecting links, however remote or obscure, with (a).

To avoid ambiguity, therefore, the distinction between "contacts" and "non-contacts" should be expanded into a distinction between (a) direct contacts (known), (b) indirect contacts (known), (c) unknown contacts (direct or indirect) and (d) persons who have not been contacts either directly or indirectly.

Turning now to the practical problem, what is wanted is to ascertain the distribution of the meningococcus in the general population, and for that purpose the population has to be "sampled." It is already known that a good many people who have been associated, directly or indirectly,

with cerebro-spinal fever are carriers, but as it cannot be taken for granted that these persons are a representative sample of the general population, the "samples" must be taken from persons unconnected, so far as is known, with the disease. If it turns out that amongst these people, conveniently termed "non-contacts," carriers are few in number, the occurrence of such may possibly be explained on the hypothesis that they really belong to class (c), the "unknown contacts." But if, amongst such "non-contacts," carriers are found in considerable percentage, the hypothesis that they are really indirect but undiscovered cases of contact with the disease is of no assistance; it can neither be confirmed nor refuted, nor does it affect the main fact of practical importance that carriers are freely distributed amongst the normal population.

INTERPRETATION OF NEGATIVE RESULTS.

The incidence of meningococci in the naso-pharynx is probably very irregular, varying in different localities and in different seasons, and therefore it is not surprising that there are marked differences in the percentages of "positives" found by different observers. And, apart from irregularities in the incidence of the cocci, it must also be generally recognised that the conditions under which the swabs are taken and investigated influence the results very materially. In taking the swab, avoidance of contamination with the common bacteria of the mouth is particularly important, because, as recently shown by Colebrook (*Lancet*, Nov. 20th, 1915), and Gordon (*British Med. Journ.*, June 17th, 1916), certain organisms commonly present in the mouth inhibit the growth of the meningococcus. In plating out the swabs, a liberal supply of medium is necessary in order to allow the development of discrete colonies, since the meningococcus, unlike hardier organisms such as the diphtheria bacillus, will either not grow at all or will not form recognisable colonies if it is surrounded and overrun by a confluent growth of other organisms. In preparing the medium for primary culture, enrichment with some adjuvant such as serum or ascitic fluid is necessary to promote the growth of naso-pharyngeal meningococci. The necessity for this has always been recognised by most bacteriologists, and recent investigations have served to emphasise its importance¹. And, above all, plenty of time and care are necessary at every stage in the process.

¹ It must not be assumed that a medium which gives a good primary culture of cerebro-spinal meningococci is necessarily good for naso-pharyngeal meningococci; the cerebro-spinal fluid transferred to the plate along with the former organisms is itself an adjuvant to growth.

from the taking of the swab to the searching of the plate and the examination of suspicious colonies.

The above considerations must be taken into account when interpreting negative results, as these would lose their significance if they were not obtained under conditions specially favourable to the discovery of any meningococci possibly present. And, it may be necessary to point out, cases in which the cultures are overgrown should be separately recorded as such, and should be eliminated from the figures on which the percentage of positives is based.

NON-CONTACT CARRIERS AT ST BARTHOLOMEW'S HOSPITAL.

In continuation of the work recorded in my previous report (pp. 442-4), Mr C. E. West, F.R.C.S., Aural Surgeon to St Bartholomew's Hospital, took naso-pharyngeal swabs from two further series of out-patients, and sent the material to the Board's Laboratory for bacteriological examination. As before, the patients were taken as general examples of hospital out-patients or convalescents, and had not, so far as could be ascertained, been in contact with any cases of cerebro-spinal fever. The same technique of investigation was observed as in the previous year's work. The results were as follows:—

I.—*Cultural Tests of 100 Naso-pharyngeal Swabs taken* *Jan. 10th—24th, 1916.*

Age period	Males		Females		Totals (Male and Female)	
	Positive	Negative	Positive	Negative	Positive	Negative
0-5 years	0	6	0	5	0	11
5-10 „	1	10	0	8	1	18
10-20 „	0	14	1	17	1	31
20-40 „	2	10	1	10	3	20
Over 40 „	1	6	3	5	4	11
	<hr/> 4	<hr/> 46	<hr/> 5	<hr/> 45	<hr/> 9	<hr/> 91

In two of the nine positives, the original plates yielded pure cultures of meningococci; in four, the colonies of meningococci were numerous or moderately numerous; and in the remaining three the colonies were scanty.

II.—*Cultural Tests of 100 Naso-pharyngeal Swabs taken
April 6th—June 5th, 1916.*

Age period	Males		Females		Totals (Male and Female)	
	Positive	Negative	Positive	Negative	Positive	Negative
0-5 years	0	2	0	1	0	3
5-10 „	0	5	2	3	2	8
10-20 „	8	12	3	10	11	22
20-40 „	8	7	5	8	13	15
Over 40 „	6	8	2	10	8	18
	<hr/> 22	<hr/> 34	<hr/> 12	<hr/> 32	<hr/> 34	<hr/> 66

In one of the thirty-four positives, the original plates yielded a pure culture of meningococci; in twenty-five, the colonies of meningococci were numerous or moderately numerous; and in the remaining eight the colonies were scanty.

Two swabs, not included among the above, are interesting. One was taken on May 4th from a female patient, aged 53, suffering from Eustachian catarrh, and yielded numerous colonies of meningococci; on January 13th a swab from the same person had given a practically pure culture of meningococci. On May 4th a swab taken from a female patient, aged 52, suffering from pharyngitis, gave moderately numerous colonies of meningococci; a previous swab, taken on January 20th, had been found positive, with numerous colonies of meningococci.

On setting out the whole of the St Bartholomew's Hospital results in successive batches of 100, the data are:—

Period	Number of Positives
March 29th—April 19th, 1915	20
April 19th—May 6th, 1915	7
May 6th—June 7th, 1915	6
June 7th—June 24th, 1915	7
*June 24th—July 22nd, 1915	†11
January 10th—January 24th, 1916	9
April 6th—June 5th, 1916	34
* Only 80 cases examined.	† Percentage.

CARRIERS IN KENT, CAMBRIDGE, AND NORWICH.

Dr Scott has examined both contacts and non-contacts in East Kent, and has reported his results on pp. 240-5; and Captain Ponder has investigated the non-contact populations of Cambridge and Norwich (pp. 247-280).

It will be seen from Dr Scott's report that a high percentage of persons carrying undoubted meningococci was found amongst non-contacts as well as amongst contacts; and Captain Ponder has shown that similar organisms were found in high percentage amongst non-contacts, including healthy workpeople, at Cambridge and Norwich. These results are in conformity with the figures obtained for London.

VALUE OF SIMPLE AGGLUTINATION TESTS AS AN AID TO
DIAGNOSIS.

IS THERE A PSEUDO-MENINGOCOCCUS?

As I showed in my last report, many investigators have used the prefix "pseudo" without adequate justification. Apparently they took it for granted that genuine meningococci would not be found in the throats of non-contacts, and some of them failed to appreciate the difficulties of serological diagnosis which arise from the fact that strains of undoubted meningococci are not necessarily agglutinated by the particular serum employed. Hence a non-contact strain, though indistinguishable culturally from the meningococcus, was branded with some such prefix as "pseudo" for the insufficient reason that it did not tally with certain standard strains in serological reactions. But since then it has been recognised that different strains behave differently towards different sera and exhibit a tendency to serological grouping. This is an important advance towards accurate identification and classification, and I note that, in view of this fact, the Medical Research Committee's Report¹ expresses the hope (p. 19) "that the terms 'para-' and 'pseudo-meningococcus' will in time be dropped."

But, whilst condemning arbitrary usage of the designation "pseudo," the above considerations are not enough to dismiss the important practical question:—Does the naso-pharynx harbour organisms which, though "meningococcus-like" and perhaps botanically related to meningococci, are incapable of producing meningitis?

In raising this question the position of organisms resembling the cholera vibrio may be considered as analogous. The search for the meningococcus in the human naso-pharynx may be compared to the search for the organism of cholera in an Indian water-tank, wherein there are frequently to be found vibrios which are "cholera-like" but are not true cholera. Greig, for example, has investigated a large

¹ *Report of the Special Advisory Committee upon Bacteriological Studies of Cerebro-Spinal Fever during the Epidemic of 1915.*

number of such cholera-like vibrios and has found¹ that they resembled true cholera culturally, but were not agglutinated by a high titre cholera serum, and did not produce agglutinins for the standard cholera vibrio. The last test he regards as important, because, as he has explained in a previous article², the true cholera vibrio may lose its agglutinability but does not lose its agglutinogenic capacity even if exposed to the action of water for a long period.

Reverting to the meningococcus, the parallel question will be:—

Are the organisms commonly found in the non-contact naso-pharynx distinguishable from cerebro-spinal meningococci in that they do not agglutinate well with any sera prepared by the latter and that they are incapable of producing sera which will agglutinate the latter?

On reference to the work of the Board's investigators it is clear that the great majority of non-contact meningococci do not answer to this description.

Dr Griffith has compared the agglutinability of 66 cerebro-spinal strains and 86 non-contact naso-pharyngeal strains with six sera prepared with spinal strains and has found (p. 132) that 94 per cent. of the former and 72 per cent. of the latter were agglutinated up to 400 or over with one or more of these sera. On preparing monovalent sera with six of his naso-pharyngeal strains, he found (p. 137) that these latter sera showed:—“(a) good agglutination with cerebro-spinal meningococci, though usually short of full titre; (b) more uniform influence on Group I strains; (c) agglutination of some of the Group II strains to half full titre.” Supplementing these data with observations on a batch of more recently isolated cerebro-spinal strains, he has found (p. 190) that 19 out of 23 of these were agglutinated up to 400 or over by the serum prepared from his naso-pharyngeal strain NP 44 (titre 1 : 800).

Dr Scott examined 71 naso-pharyngeal strains. Of these (p. 230), 44 agglutinated with his Group II sera (30 up to full titre, 9 to 1,000; and 5 to 500; one of the last 5 also went up to 500 with a Group I serum). Of the remaining 27, 14 agglutinated with Group I sera (2 up to 1,500, 2 up to 1,000, and 10 up to 500). As regards agglutinogenic capacity, 8 of these 14 naso-pharyngeal strains produced sera agglutinating certain cerebro-spinal members of Group I. Agglutinogenic capacity of naso-pharyngeal strains resembling cerebro-spinal members of Group II was not investigated.

¹ The Serological Investigation and Classification of Cholera-like Vibrios isolated from water in Calcutta. *Indian Journ. of Med. Research*, April, 1916.

² *Ibid.*, Jan., 1916.

Captain Ponder, who did not find that his cerebro-spinal strains were clearly separable into two main groups, tested the agglutinability of 94 non-contact naso-pharyngeal strains with sera prepared from cerebro-spinal strains, and found that 74 per cent. of these non-contact strains "gave evidence of relationship to the meningococcus in virtue of their agglutination reactions" (p. 280). He tested the agglutinogenic capacities of two of his naso-pharyngeal strains, Nos. 108 and 235, towards 16 spinal strains; 13 of these were agglutinated to 400 or over with the serum prepared from the former (titre about 1 : 400), and 9 of these 13 were also agglutinated to 400 or over by NP 235 serum (titre about 1 : 800).

COMPARISON BETWEEN THE NON-CONTACT MENINGOCOCCUS AND
THE PNEUMOCOCCUS "CARRIED" BY NORMAL INDIVIDUALS.

Whilst recognising that there is no evidence of a valid analogy between water vibrios which are merely "cholera-like" and prevalent strains of non-contact meningococci, it might be urged that a better analogy is provided by recent serological work on the pneumococcus, the outcome of which is to suggest that the pneumococci commonly met with in the mouths of normal individuals, though genuine pneumococci and not merely "pneumococcus-like," are distinguishable from the majority of pneumococci which have been responsible for lobar pneumonia or other acute infection. As this is an important suggestion, which was raised a year ago in the Medical Research Committee's Report, it calls for consideration in the light of laboratory data.

In 1910, Neufeld and Haendel¹ called attention to the occurrence of pneumococci which did not agglutinate with standard sera, and expressed the opinion that extensive enquiry ought to be made into the prevalence of special types of pneumococci and into the occurrence and distribution of atypical strains.

Recognising the importance of the problem raised by Neufeld and his associates, Dochez and Gillespie (1913)² attempted to form a biological classification of pneumococci by means of immunity reactions. They investigated the pneumococci derived from 74 cases of typical lobar pneumonia and grouped them as follows:—

¹ *Arb. aus d. Kaiserl. Gesundheitsamte*, XXXIV. 293.

² *Journ. American Med. Assoc.*, LXI. 727.

Group	No. of Cases	Percentages
I.	35	47
II.	13	18
III. (<i>mucosus</i>)	10	13
IV. (heterogeneous)	16	22

As the Group III organism, the *Pneumococcus mucosus*, is distinguishable culturally from the pneumococci in the other groups, it does not concern the meningococcus problem. Omitting this group, there are left 64 cases, of which 35 (55 per cent.) fall into Group I, 13 (20 per cent.) into Group II, and 16 (25 per cent.) into Group IV. Groups I, II and IV were indistinguishable morphologically and culturally, but serological tests gave the following results. A Group I serum protected white mice against all Group I strains, but not against any strains of II or IV, and this serum also agglutinated all Group I strains, but no strains of II or IV. Similarly, *mutatis mutandis*, with Group II. Group IV is not a group in the same sense as the other two. It is the residue, and comprises organisms which all differ serologically from each other as well as from those in I and II. "This group comprises a number of distinct varieties of pneumococcus which cannot be related to one another by immunologic reactions. Culturally they are true pneumococci, and manifest all the common characters of pneumococcus." The authors go on to suggest that the other groups comprise the "fixed races," which "are more highly parasitic and are never very far removed from a condition of pure parasitism, whereas the heterogeneous strains may be representatives of the types of pneumococcus found in the normal mouth, and consequently more likely to have undergone environmental changes."

In continuation of the above work, Dochez and Avery (1914 and 1915)¹ examined the pneumococci from 71 additional cases of lobar pneumonia and classed them as follows:—

Group	No. of cases	Percentages
I.	21	30
II.	28	39
III. (<i>mucosus</i>)	6	8
IV. (heterogeneous)	16	23

¹ *Journ. Exper. Med.*, xxi. 114 and xxii. 105.

Omitting Group III and combining the remaining data with those previously recorded, out of 129 cases of lobar pneumonia, there were 56 (43 per cent.) in Group I, 41 (32 per cent.) in Group II, and 32 (25 per cent.) in Group IV. The authors noted that the case mortality was lower in Group IV than in any of the other groups.

They also studied the pneumococci in convalescents from pneumonia, in healthy contacts, and in the sputum of non-contacts. In convalescents they found that generally the "fixed types," I-III, disappeared, and were replaced by IV, though sometimes a convalescent was a carrier of a "fixed type" for a long time. In healthy contacts the "fixed types" were often found. About 60 per cent. of the mouths of normal persons (not contacts) yielded pneumococci, but these organisms all belonged to Group IV.

With reference to the pneumococci in each of their four groups the authors say that "up to the present time we have observed no tendency of these organisms to lose their specific characters, nor have we observed a change of one type into another."

Cole (1915)¹ gives some further information as to the relative virulence of the four groups in cases of pneumonia, viz.:

Group	Cases	Deaths	Mortality Percentage
I.	28	7	25
II.	25	9	36
III.	17	8	47
IV.	33	2	6
	<hr/> 103	<hr/> 26	<hr/> 25

He also quotes the corresponding mortality statistics from the Pennsylvania Hospital, which show 29 per cent. for Group I, 27 for Group II, 67 for Group III, and 11 for Group IV.

Stillman (1916)² summarises for the four years 1912-13 to 1915-16 the types of pneumococci isolated from cases of lobar pneumonia admitted to the hospital of the Rockefeller Institute:—

Type	Number of cases	Percentage
I.	105	33·54
II.	99	31·62
III.	35	11·18
IV.	74	23·64

¹ *New York Med. Journ.*, Jan. 2, 1915, p. 1

² *Journ. Exper. Med.*, xxiv. 651.

The above data raise a clear issue. Do non-contact meningococci resemble the American Group IV pneumococci?

As regards pathogenicity, the Group IV pneumococcus is said to be responsible for about 25 per cent. of the cases of lobar pneumonia, though these cases have a relatively low death-rate. If the non-contact meningococcus bears a similar relationship to meningitis, it is obviously very far from being a harmless saprophyte.

Serologically, Group IV pneumococci differ markedly from each other and show no relationship to Groups I or II. The data quoted above as to the serological reactions of non-contact meningococci and their relations to meningococci of cerebro-spinal origin show that it is impossible to make a serological subdivision of meningococci which would place the non-contact strains in an independent group, resembling the Group IV pneumococci as regards individual differences in agglutinability and agglutinogenic capacity, and differing from the other groups of pneumococci which are said to be found only in pneumococcal infections or in contacts therewith.

It would, however, be unsafe to draw the conclusion that there is no real parallel between the non-contact carrier of the pneumococcus and the non-contact meningococcus carrier. On the contrary, there appears to be an interesting, and probably a very important, parallelism between the two conditions; and the inference I would prefer to draw from the literature I have quoted is that the American theory needs independent re-investigation in this country and cannot, at present, be regarded as permanently established¹. Perhaps the American investigators are already beginning to discover this.

In the recent article by Stillman, to which I have referred above, there is the significant statement that, though Types I and II are not found in the normal mouth except in the case of contacts, recent studies have shown that Type III is "fairly common in the mouth flora of healthy individuals and infections with organisms of this type may be autogenic in nature." Type III, he goes on to say, was found in 44 out of 398 normal persons (23.4 per cent.) whilst Type IV was found in 58.5 per cent. There was no serological difference between non-contact Type III and pneumonic Type III; and it was found that the former

¹ F. S. Lister has found that in the pneumonia of South African miners there are at least four groups of pneumococci in addition to those recognised by the Americans. (The South African Institute for Medical Research. No. VIII. *An Experimental Study of Prophylactic Inoculation against Pneumococcal Infection in the Rabbit and in Man*. Published by the Institute, Oct. 1st, 1916.)

might persist in the normal mouth for a long time. Here one may remark that the wide distribution of Type III (*Pneumococcus* or *Streptococcus mucosus*) in normal individuals is not a new discovery. It is mentioned, for example, by Lingelsheim (1912)¹ as a well-known fact that this organism is a not uncommon inhabitant of the upper respiratory tract.

Since Type III, the most virulent of the so-called "epidemic" types, turns out to be an organism which quite commonly lives the existence of a harmless saprophyte, it is difficult to understand why the same should not be true of Types I and II. Further research is needed on this point. Perhaps a fuller investigation of the miscellaneous collection known as "Type IV" will throw some light on the question. In the earlier days of meningococcus work, before the importance of serological differences was appreciated, a great many strains were found which did not respond to the particular sera with which they were tested and, on this account, might have been relegated to a miscellaneous scrap-heap similar to the American Type IV; but, now that more suitable sera have been obtained, the atypical residue which fails to respond to one or other of these has been greatly diminished. Similarly it may be possible to find pneumococcal sera which will rearrange and possibly link together the pneumococcal groups now known as I, II and IV. And, in this connection, another matter for consideration will be the possibility that bacterial antigen may be modified in the human tissues.

DIAGNOSTIC VALUE OF ABSORPTION OF AGGLUTININ.

COMPARISON BETWEEN INTESTINAL BACTERIA AND MENINGOCOCCI.

Certain investigators have claimed that between the two organisms *B. suipestifer* and *B. paratyphosus* (B), which are indistinguishable culturally and often agglutinate well with the same serum, a clear distinction can be brought out by resorting to the test for absorption of agglutinin. Their observations naturally raise the question whether the same method might not serve to distinguish between "non-contact" meningococci and strains of cerebro-spinal origin. I propose therefore first to state the evidence in support of the view that *B. suipestifer* and *B. paratyphosus* (B) are distinguishable by absorption of agglutinin and then to call attention to the results of this test when applied to meningococci obtained from different sources.

¹ Kolle u. Wassermann's *Handbuch der path. Mikroorg.*, 2nd Ed., iv. 498.

B. suipestifer and *B. paratyphosus* (B).

Boycott (1906)¹ in the course of an enquiry into the bacteriology of paratyphoid fever and the diagnostic value of serological tests, discussed the method of differentiation by absorption of agglutinin. As subsequent writers include Boycott amongst the investigators who are able to distinguish *B. paratyphosus* (B) from *B. Aertryck* (generally agreed to be identical with *B. suipestifer*) by the absorption method, it will be useful to select from Boycott's article the records of his experiments which have a bearing on this point.

(1) The serum of a rabbit immunised with *B. Aertryck* gave a titre of 1 : 2,000 for a strain of *Aertryck* and the same for a strain of *B. paratyphosus* (B). Absorption with *Aertryck* completely removed agglutinin for both strains; whereas absorption with *paratyphosus* (B) removed all agglutinin for itself but removed none of the agglutinin for *Aertryck*.

(2) The serum from a patient named "Barkley" was tested on two occasions. On the first, absorption with a strain of *paratyphosus* (B) removed all agglutinin both for this organism and for a strain of *Aertryck*, whilst absorption with *Aertryck* removed agglutinin for itself but not for *paratyphosus* (B). On the second occasion, when the titre of the Barkley serum was 1,000 for a strain of *Aertryck* and over 5,000 for a strain of *paratyphosus* (B), a single absorption with *Aertryck* removed the agglutinin for itself but did not affect the agglutinin for *B. paratyphosus* (B); absorption with *B. paratyphosus* (B) removed the agglutinin both for itself and for *Aertryck*, but only after treatment three times; the first and second doses of absorbing culture failed to remove agglutinin for either organism.

(3) The serum from a patient named "Valérie" lost its agglutinin for both *Aertryck* and *paratyphosus* (B) when absorbed with the latter organism; but, when absorbed with the former, the agglutinin was retained for *paratyphosus* (B) and lost for *Aertryck*.

The standard strain of *B. paratyphosus* (B) which Boycott used was "*Schottmüller B*, original strain (1901)": and his *Aertryck* was a strain isolated by Prof. van Ermengem from an outbreak of food poisoning.

Bainbridge (1909)² used the absorption of agglutinin test for differentiating between *B. paratyphosus* (B) and the two indistinguishable organisms, *B. Aertryck* and *B. suipestifer*. The dilution of the serum used for absorption varied from 1 : 10 to 1 : 50 but was usually 1 : 20

¹ *Journ. of Hygiene*, vi. 33.

² *Journ. of Path. and Bact.*, xiii. 443.

or 1 : 40. It was found that differentiation was most clearly brought out "by comparing the agglutination limits of the serum for these bacilli after one or more absorptions with a moderate amount of bacilli."

A *B. Aertryck* serum (titre 1 : 5,000 for the homologous organism and also for a strain of *B. paratyphosus* (B)), when absorbed with *B. Aertryck*, failed to agglutinate either organism in 1 : 200; when the serum was treated with *B. paratyphosus* (B), the first absorption reduced the paratyphoid agglutination limit to 200 and the limit for *Aertryck* to 1,000, a second absorption brought down the former limit to below 100 and the latter to 2,000, a third absorption produced no further change.

A *B. paratyphosus* (B) serum (titre 1 : 5,000 for the homologous strain; 1 : 1,000 for a strain of *B. Aertryck* and the same for a strain of *B. suipestifer*), when absorbed with *B. paratyphosus* (B) failed to agglutinate all three organisms above 200; when the serum was treated with *B. Aertryck*, the first absorption reduced the paratyphoid agglutination limit to 4,000 and the limits for *Aertryck* and *suipestifer* to below 200; a second absorption reduced the paratyphoid limit to 2,000 and the limits for the other two organisms to below 100; a third absorption brought the paratyphoid limit down to 1,000.

A *B. suipestifer* serum (titre 1 : 10,000 for the homologous strain and also for a strain of *B. paratyphosus* (B)), when absorbed with *B. suipestifer*, failed to agglutinate either organism above 100; when absorbed with *B. paratyphosus* (B), agglutinin for this strain fell below 100 but agglutinin for *suipestifer* was retained at 10,000.

In the above experiments apparently the same three strains were used for producing the sera. for absorption, and for determining the titres of the sera before and after absorption.

Bainbridge has tabulated a further series of absorption experiments in which he used five sera (two *Aertryck*, two *paratyphosus* (B), and one *suipestifer*), absorbed each of these with an *Aertryck*, a *paratyphosus* (B), and a *suipestifer* strain, and determined the titre before and after absorption with three strains bearing the same designations (?actually the same strains). The results were in accordance with those obtained in the former series of experiments. Apparently some of the strains used in the second series, for producing the sera. for absorption, and for agglutination before and after absorption, were the same as those used in the first series.

Two of Bainbridge's standard strains, one *B. paratyphosus* (B) and one *Aertryck*, were the same as those used by Boycott. His two standard

suipestifer strains were "(a) the laboratory strain (Král); (b) a strain obtained from Prof. Wassermann."

O'Brien (1910)¹ isolated from an epizootic in guinea-pigs an organism belonging to the food-poisoning group. When tested with an *Aertryck* serum, this organism agglutinated up to full titre (1 : 5,000), as, also, did a strain of *B. paratyphosus* (B); absorption with *B. paratyphosus* (B) removed all agglutinin for itself but left agglutinin up to 1 : 2,000 for both *Aertryck* and the guinea-pig organism. When tested with a *paratyphosus* (B) serum, the guinea-pig organism agglutinated up to full titre (1 : 2,000), as, also, did a strain of *Aertryck*; absorption with *Aertryck* removed all agglutinin for itself and also for the guinea-pig organism, but left agglutinin up to 1 : 500, for *paratyphosus* (B).

Bainbridge and Dudfield (1911)² described an outbreak of acute gastro-enteritis caused by *B. paratyphosus* (B). Simple agglutination tests failed to discriminate between this organism and *suipestifer*, but the application of the absorption method brought out a sharp distinction in favour of the former bacillus.

Bainbridge and O'Brien (1911)³ investigated the value of the absorption method for grouping a certain number of strains which agglutinated well with both *paratyphosus* (B) and *suipestifer* sera.

The material they used consisted, in the first place, of certain standard strains, all of which were well authenticated. These strains were:—(a) Schottmüller's original strain of *B. paratyphosus* (B), which had previously been tested by Boycott in 1906 and by Bainbridge in 1909; (b) a *B. paratyphosus* (B) strain from McWeeney, apparently the one used by Bainbridge and Dudfield in 1911 in the investigation mentioned above; (c) a *suipestifer* strain designated "Laboratory (Král)," which had been used by Bainbridge in 1909 and by Bainbridge and Dudfield in 1911; (d) a *suipestifer* strain from Uhlenhuth; (e) a *suipestifer* strain from Wassermann which had been used by Bainbridge in 1909.

Secondly, 24 laboratory strains of the paratyphoid or food-poisoning group were collected from various sources and were compared with the five standard strains.

Referring to the technique of absorption experiments, the authors stated that it was possible by the addition of very large amounts of a heterologous bacillus to remove some of the homologous agglutinin from a serum, but they had not succeeded in removing all the homologous agglutinin in that way. "The difference between the amount of heterologous bacilli which must be added to serum to absorb only the hetero-

¹ *Journ. of Hygiene*, x, 231.

² *Ibid.* xi, 24.

³ *Ibid.* x, 68.

logous agglutinin, leaving the homologous agglutinin intact, and that necessary to absorb much of the homologous agglutinin, is so large that errors cannot occur if reasonable care is taken." For example, they used 2 c.c. of a 1 : 10 dilution of *suipestifer* serum (titre, 1 : 20,000 for the homologous strain, 1 : 5,000 for a strain of *B. paratyphosus* (B)); absorbed with two agar slopes of *paratyphosus* (B), its agglutination limit was reduced to below 100 for *paratyphosus* but remained at 20,000 for *suipestifer*, and the latter limit remained unaltered when absorption was made with eight agar slopes. The authors observed that it was preferable to measure the maximum titre of agglutination with the serum after absorption rather than merely to observe the agglutination at one or two dilutions.

The authors have recorded in full the essential details of their laboratory work; as the results were uniform, a brief summary will suffice. The absorption tests divided their five standard strains into (1) (a) and (b), which conformed to their *paratyphosus* standard, and (2) (c), (d), and (e), which conformed to their *suipestifer* standard. Absorption tests also divided their 24 additional strains into (1) those conforming to their *paratyphosus* standard and (2) those conforming to their *suipestifer* standard, with the following exceptions—one (No. 19) agglutinated with unabsorbed *suipestifer* serum up to 10,000, and the titre after absorption with *paratyphosus* fell to below 100, but the same organism gave 5,000 with unabsorbed *paratyphosus* serum and after absorption with *suipestifer* the titre fell to below 200; of two other strains, one (No. 16) was a poor agglutinator and the other (No. 20) was practically inagglutinable with the two standard sera.

Meningococci.

For the details of the work on this subject I must refer to the reports by Dr Griffith, Dr Scott, and Captain Ponder.

Dr Griffith (p. 129) has found that 62 of his 86 naso-pharyngeal strains agglutinate up to 400 or higher with one or more of his Group II sera prepared from spinal strains. He has tested the absorptive capacity of 33 out of these 62 strains, and has found that they all exhaust the homologous agglutinin from one or more of his spinal sera. This result, he considers, is sufficient to justify the conclusion that the remainder of these 62 strains would be found to absorb the homologous agglutinin from some Group II spinal serum. Of his remaining 24 strains, some, as he has shown in detail on pp. 161–4, are related in absorptive capacity to Group I strains of spinal origin.

Dr Scott has found (p. 246) that 58 of his 71 naso-pharyngeal strains afford proof of complete serological identity (including identification by the absorption test) with known pathogenic strains.

Captain Ponder (p. 280) has not tested all his strains by the absorption method, but he has taken the cultures from his last series of 100 swabs, all obtained from healthy workpeople, and has submitted to the absorption test all those which were like meningococci in simple agglutination. He only employed one serum for this purpose. He found that nine of his strains absorbed agglutinin as well as the homologous coccus, and four others absorbed it partially. On applying the absorption test to another batch of strains he obtained similar results.

VALIDITY OF DIFFERENTIATION BY ABSORPTION OF AGGLUTININ.

The results of the work on the meningococcus suffice to show that absorption of agglutinin tests do not separate "non-contact" from cerebro-spinal meningococci in the way in which such tests divided the strains of *B. paratyphosus* (B) and *B. suipestifer* which were investigated by Bainbridge and O'Brien. This lack of correspondence raises several problems which require consideration, the first question being whether the work of Bainbridge and O'Brien justifies a general statement that organisms giving the cultural reactions common to the large group of which *B. paratyphosus* (B) and *B. suipestifer* are members can be differentiated by absorption of agglutinin. This statement has been disputed by certain German pathologists, on grounds which are given in the following summary.

Objections.

Uhlenhuth, Hübener, Nylander, and Bohtz (1909)¹ maintained that *B. paratyphosus* (B) and the hog-cholera group of organisms could not be classified into separate groups either culturally or by serological tests. In this connection they discussed the contention that, though indistinguishable culturally and by simple agglutination tests, *paratyphosus* (B) and *suipestifer* were clearly separable by the adoption of Castellani's principle of differentiation by the absorption of agglutinin. This, they found, was not the case. They admitted that clear differences might be apparent if reliance were placed on a single strain as representative of each alleged group of organisms; but, when a large number of strains were used, the results of the absorption tests were so irregular that a demarcation into distinct groups became impossible. This irregularity

¹ *Arb. a. d. Kaiserlich. Gesundheitsamte*, xxx, 292.

they attributed to individual differences in the "receptor apparatus" of different strains. They based their conclusions on the results of absorption tests with a very large number of different strains and, in the present article, have taken one series of experiments as an example and tabulated their results. The table is too long to reproduce here, but it will suffice to quote some of the essential details of the experiments which it records.

Their method of conducting the tests was as follows: a rabbit serum (titre 1 : 5,000) was prepared from a human *paratyphosus* (B) strain named "Hellwig," and was diluted to 1 in 500. To 100 c.c. of this dilution was added, in the case of each strain used for absorption, the 24 hours' growth obtained on 20 agar tubes. The mixture was incubated at 37° C. for two hours, and then centrifuged until completely clear fluid was obtained. For testing the absorbed serum as to its remaining agglutinating power (dilution 1 : 500), 1 loopful of 24 hours' culture was used to 1 c.c. of fluid; the mixture was incubated at 37° C. for one hour and then kept at room temperature for 24 hours. The same procedure was adopted in each test.

When the homologous strain, "Hellwig," was used for absorption, it removed the agglutinin for itself and for the 19 other human *paratyphosus* (B) strains which were tested.

But when other strains were used for absorption of this serum uniformity of results was no longer obtained.

Absorption with "Eb.," one of the above 20 human paratyphoid strains, removed agglutinin for itself but not for "Hellwig," and with the remaining 18 strains the results were irregular, agglutinin being removed for eight, but retained for eight others, whilst with the last two the result of the test was doubtful.

Absorption with "England," another of the above 20 strains, removed agglutinin for itself, for "Eb.," for "Hellwig," and for 12 other of these strains, but failed to remove agglutinin from the remaining five, including one which gave the opposite result when the serum was absorbed with "Eb."

The "Hellwig" paratyphoid serum was then absorbed with certain strains of *suipestifer* obtained from pigs, using the same quantities of culture and the same technique in every respect as in the former experiments. The first *suipestifer* strain removed agglutinin for 18 out of 27 *suipestifer* strains, all isolated from pigs; but it also removed the agglutinin for "Hellwig" and for 12 other of the 20 human *paratyphosus* (B) strains. It failed to remove the agglutinin for "Eb.," for "England,"

and for four other strains. With the one remaining strain the result was doubtful.

A second *suipestifer* strain, from a normal pig, when used for absorption of the same paratyphoid serum, removed the agglutinin for 21 out of the 27 *suipestifer* strains; but it also removed the agglutinin for "Hellwig" and for 11 other of the 20 human *paratyphosus* (B) strains. It failed to remove the agglutinin for the remaining eight, including "Eb." and "England."

Similar irregularities in results were obtained when the serum was absorbed with other strains. *e.g.*, a strain obtained from a case of food poisoning and one isolated from a sausage. These need not be quoted in detail, as the above data suffice to support the authors' statement that the method was found to be unreliable for diagnostic purposes. Of the five absorbing strains about which I have given details, only one gave unequivocal results; and that was the one used to produce the immune serum. The other two paratyphoid (B) strains used for absorption failed to absorb agglutinin for several paratyphoid (B) strains; and the two *suipestifer* strains used for absorption, which, according to the absorption theory, should have left the paratyphoid agglutinin untouched, removed this agglutinin for more than half of the paratyphoid strains.

Are the Objections Valid?

The importance of the laboratory data, quoted above, which were recorded by Uhlenhuth and his co-workers in 1909, lies in the fact that, under identical conditions of experiment, the individuality of different strains comes out very strongly and makes its appearance in such irregular fashion that no basis is provided for a subdivision of these strains into distinct groups.

But, in the absence of fuller particulars, these results cannot be regarded as a conclusive proof that differentiation of these organisms by the adoption of Castellani's principle is impossible. No information is given as to the highest dilution in which agglutination was obtained after absorption, and there is no evidence that the quantity of culture used for absorption was the amount most favourable for enabling a group distinction to make its appearance.

For example, the paratyphoid serum (titre, 1 : 5,000) was absorbed with the paratyphoid strain "Eb." and it was found that the absorbed serum still agglutinated eight paratyphoid strains at 1 : 500. But this is a very incomplete statement of the relationship of "Eb." towards

these eight strains. Presumably, these eight strains agglutinated with the unabsorbed serum as well, or nearly as well, as the homologous strain. Did absorption with "Eb." leave their agglutination limit unaffected or only slightly affected, or did it bring that limit down somewhere to the neighbourhood of 500? As no answer to these questions is given, it is impossible to exclude the latter alternative. Furthermore, since no evidence is given to the contrary, one cannot exclude the possibility that a much smaller quantity of "Eb." culture would have been as effective, or nearly as effective, in bringing about a marked reduction of agglutinin for all the 20 paratyphoid strains.

Again, when a *suipestifer* strain was used for absorption, similar questions arise concerning its failure to remove agglutinin (at 1 : 500) for 9 out of 27 *suipestifer* strains. Possibly it effected a marked reduction of agglutinin for these nine strains; and possibly a much smaller quantity of absorbing culture would have produced very similar results on all 27 strains.

Moreover, in the absorptions with *suipestifer* strains, the objection has not been met that the use of much smaller quantities of absorbing culture might have produced relatively little loss of agglutinin for paratyphoid strains but, at the same time, well marked loss for *suipestifer* strains.

It will be noted also that the serum used for absorption was very dilute (1 : 500), and therefore more readily affected than more concentrated sera.

In considering the work of Bainbridge and O'Brien it must at once be recognised that they have investigated a considerable number of strains and, by means of the absorption method, have succeeded in almost every instance in allocating each to one of two groups, according as it conforms (*a*) to their *paratyphosus* standard or (*b*) to their *suipestifer* standard.

At first sight the fact that a considerable number of strains was used seems to dispose of Uhlenhuth's objection that division into groups is only possible when attention is confined to single strains as representative of each alleged group. The strains used for testing against the standard organisms were isolated from human or animal material sent to several different laboratories, and were therefore derived from several different and independent human or animal sources; and in most cases, presumably, it had been established in the laboratories providing these strains that they were typical representatives of the paratyphoid (B) and food-poisoning group of organisms, and were therefore suitable for

submission to the absorption test. But here an important laboratory point arises. As a matter of routine diagnosis the strains must have been identified by testing their agglutinability with standard sera, and probably these standards were established either by the original strains regarded as representative of this group of organisms (*Schottmüller* (B) and Van Ermengem's *B. Aertryck*) or by strains proved to be identical with these. Many investigators have found that a "typical strain," i.e. one which conforms to their standards, can be differentiated by simple agglutination alone, since, when tested with good sera, its agglutination limit is much higher for *paratyphosus* serum than for *suipestifer* serum or *vice versa*. It therefore seems fair to raise the question whether the majority of the strains sent to Bainbridge and O'Brien had already been selected as "typical," in the sense defined above, or whether they were random samples and truly representative of the range of variation of *B. paratyphosus* (B) and of *B. suipestifer* which may occur in nature. Bainbridge and O'Brien give no information on this point. They have, however, found that, when their 24 strains were tested with sera prepared by injection of living cultures, before resorting to absorption "some indication was revealed of the existence of two types of bacilli." Referring to their tabulated record of these experiments, it is seen that two of their 24 strains may be omitted as being poor agglutinators with both the *suipestifer* and the *paratyphosus* serum, and that each of the remaining 22 showed a clear difference between its agglutination limit with the one serum and its limit with the other. This difference was never less than 2:1 (e.g. 20,000 as against 10,000 or 10,000 as against 5,000) and occasionally it was much greater. These results are strong indication of initial conformity to standard: and it will be found that the diagnosis thus indicated by agglutination alone is confirmed in 21 out of the 22 strains by the authors' subsequent absorption tests. The exceptional case is No. 19 which on simple agglutination, reached 10,000 with *suipestifer* serum as against 2,000 with *paratyphosus* serum: the absorption results, however, are ambiguous, as absorption of *suipestifer* serum with *paratyphosus* reduced the agglutinin for this strain from 10,000 to below 100, whilst absorption of *paratyphosus* serum with *suipestifer* also effected a marked reduction in agglutinin for the strain (from 5,000 to below 200).

On this view, the fact that all the strains investigated had been well authenticated may have been a disadvantage, because it may mean that they had been selected as "typical" owing to their conformity with one or other of two well-known serological criteria. If this was the case,

Uhlenhuth's objection against the absorption method has not been refuted. On the other hand, supporters of the absorption theory may regard Uhlenhuth's laboratory data as inconclusive. It will be best, therefore, to leave the question as still unsettled. Evidently these intestinal organisms show a tendency to serological grouping, just as meningococci do, and they may possibly be capable of subdivision into two large groups supplemented by a number of smaller ones. But, as the groups have not yet been fully worked out, one cannot take it for granted that the members of some of them will be exclusively "suipestifer" and the members of others exclusively "paratyphoid."

These considerations of laboratory detail are important because they have a direct bearing on a general question of bacteriological classification, which concerns the meningococcus and many other organisms, in addition to *paratyphosus* (B) and *suipestifer*¹.

How much stress ought to be laid on individual peculiarities of particular strains? There can be no doubt that in many, if not in all, widely distributed groups of organisms such peculiarities do exist; and this fact is usually brought into prominence whenever the stimulus of research leads to minute examination of a large number of organisms belonging to the same class. Then the difficulty of standardisation arises. The obvious course is to begin with a particular strain as a provisional standard and see how many other strains coincide more or less completely with this, in agglutinating with and absorbing agglutinin from the serum produced by the standard strain. If there remains a residue of aberrant strains, a second provisional standard is selected from these, and it is ascertained how much of the residue can be grouped under this second standard. To the strains, if any, which refuse to fall into the second group a similar process is applied, and so on, until all the strains are accounted for.

This method is unimpeachable if it is recognised as being no more than a preliminary orientation; but it is fallacious if it is taken as providing a final classification. Suppose, for example, the orientation method gave three groups, the respective standard strains being A, B, and C. One cannot take it for granted that all the strains in Group I are identical with A in agglutinogenic and absorptive capacities, nor that the same holds good for Groups II and III; but without such identity

¹ It is in view of this general question that I have discussed the significance of absorption experiments with food-poisoning organisms; the question of what is the best way to classify these particular organisms would involve consideration of many matters, in addition to absorption experiments, and does not come within the scope of this report.

the grouping would not be justified, since the selection of other strains as standards would then give different groups. The true standard is that which represents what exists in nature, and therefore must recognise such individual differences as occur; a standard which ignores these would be arbitrary, artificial, and not truly representative.

To put the same considerations in a more technical form, absorption is supposed to aid classification by bringing out a distinction between specific and non-specific agglutinin; the value of the absorption method must therefore be discussed in relation to theories as to the specificity of agglutinin.

PRINCIPLES DETERMINING DIFFERENTIATION OF AGGLUTININS.

I. BACTERIA OF DIFFERENT SPECIES.

If the agglutinin for each species were quite distinct from the agglutinins for the rest, the matter would be very simple. For example, a serum containing the agglutinins *a, b, c* would be due to mixed infection with the three species A, B, and C; and each species, as shown by Castellani, would absorb its own agglutinin from the serum, leaving the other agglutinins intact.

But it very often happens that there is a partial overlapping, to greater or less degree, of agglutinins produced by bacteria which are recognised, from their general biological characteristics, as belonging to distinct species. On Durham's hypothesis, this fact would be explained by assuming that agglutinin consists of several different components. Thus:

Species A may produce agglutinin *a b c d e*.

Species B may produce agglutinin *a b f g h*.

Species C may produce agglutinin *b c i k l*.

Accepting this hypothesis, provisionally, the specific agglutinins would be contained amongst the components *d e, f g h*, and *i k l*, respectively, whilst the corresponding non-specific agglutinins would be represented by *a b c, a b, b c*. This distinction would again be demonstrable by Castellani's method; *e.g.* absorption of an A serum with a B strain would remove all agglutinin for species B but would leave agglutinin practically intact for species A.

This method has often been applied as an aid to diagnosis. For example, an unknown culture X is agglutinated both with serum A and serum B. Serum A is then absorbed with culture X and the absorbed

serum is tested upon a known strain of species A. If it is found that X has removed the agglutinin for the known strain, X is regarded as belonging to species A. If this is not the case, a similar test with B serum and a known B strain may show that X belongs to species B.

The validity of this method is widely recognised in cases where a positive result is obtained, *i.e.* when the unknown strain removes specific agglutinin for either A or B.

It is to be noted, however, that if the result is negative, *i.e.* neither strain A nor strain B fails to agglutinate with the serum absorbed by X, it is not justifiable to conclude that X belongs to a third species different from both species A and species B, because a particular strain does not necessarily absorb agglutinin for all members of the same species. In such a case the absorption test would give no information of diagnostic value.

This important fact that strains of the same species may differ from each other in absorptive capacity is well illustrated by the work of Meinicke, Jaffé and Flemming (1906)¹. They tested the absorptive capacities of 47 cholera strains which were all typical and all agglutinated well, and about equally, with a standard cholera serum; this serum, which had been prepared from one strain, had no effect on "cholera-like" vibrios. Marked differences were found. Some strains absorbed agglutinin for the whole or the majority of the 47 strains; but others only removed agglutinin for a relatively small number, and in this respect they exhibited a selective action, *i.e.* the strains picked out by some absorbing strains were not the same as the strains picked out by others. This selective action was qualitative and not merely quantitative, because a strain which only removed agglutinin for a few strains could not be made to remove agglutinin for more by repeating the absorption. According to absorptive capacity, their 47 strains might be divided, roughly, into five different groups, but the demarcation of these groups was not always sharply defined, and within some of the groups a subdivision might be made. The authors thought that if the number of their strains had been larger the number of groups would probably have increased, but they fully recognised that attempted grouping of cholera strains in accordance with absorptive capacity would be devoid of practical interest.

This irregularity of absorptive capacity is particularly significant, because the cholera vibrio is remarkably specific in agglutinability and agglutinogenic capacity. With other species which are less uniform in

¹ Ueber die Bindungsverhältnisse der Cholera-vibrien. *Zeitschr. f. Hyg.*, LII. 416.

the two latter respects, it is still less likely that diagnostic significance can be attached to irregularities in capacity for absorption.

II. POSSIBLE SUB-GROUPS OF ONE SPECIES.

Whilst the absorption method often gives positive results in determining the species to which an organism belongs, its applicability to the sub-grouping of members of one species, which coincide in simple agglutinability, is another matter. In the former case, its utility consists in eliminating the ambiguity caused by the overlapping of two different sera which contain heterologous as well as specific agglutinin. In the latter case, it is not a question of distinguishing specific from heterologous or accidental agglutinin, but of emphasising those characteristics of the sub-groups which are not common to the species as a whole. Thus, borrowing Durham's conception of the multiple components of agglutinin, the sub-groups of a species may behave as follows:

- (a) Group I may produce agglutinin $abcde$.
- Group II may produce agglutinin $defgh$.
- Group III may produce agglutinin $deikl$.

The special characteristics of each group would be contained amongst the components abc , fgh , ikl ; the agglutinin common to the whole species would be de . This distinction, as in dealing with organisms of different species, would be brought out by the absorption method, but the nature of the distinction would be very different. Here, de , which is common to the three groups, is essential; it is not heterologous and cannot be eliminated as unimportant. Its presence is in no way comparable to the accidental overlapping by abc , ab , and bc , in the three different specific sera which I have figured above (p. 89).

I wish to emphasise this last point, because much confusion has been caused by loose usage of the terms "specific" and "non-specific." Admittedly, bacteriological "species" are more or less ill-defined, but there need be no practical difficulty on that score. For example, gonococci and meningococci are quite sufficiently different to be called different species; differentiation, by absorption, of a gonococcus and a meningococcus which are agglutinated by the same serum is a specific differentiation. On the other hand, cholera vibrios are all sufficiently alike to be included in one species, and the same may be said for typhoid bacilli; differentiation, by absorption, of two cholera or two typhoid strains which agglutinated with the same serum would be a minor

distinction, possibly affording a basis for sub-grouping, but not invalidating the evidence of specific relationship afforded by the simple agglutination test.

I have taken the simplest case first, where the sub-groups possess some agglutinins in common and thereby show their relationship to the species as a whole. Here, as Meinicke and his colleagues have shown, sub-grouping is possible by means of the absorption test, but in their experience with strains of cholera it seemed to be of minor importance and in no way corresponded with differences of virulence. Instead of providing a useful basis of classification, the differences in absorptive capacity which they demonstrated proved, in their opinion, that "the Castellani test could no longer be regarded as an infallible criterion."

Then there is another possibility, in the sub-grouping of some species of bacteria, where the sub-grouping is equally simple but of a different kind, being based on the absence of serological affinity between the groups, as shown by simple agglutination tests alone. Thus:

- (b) Group I may produce agglutinin *a b c*.
- Group II may produce agglutinin *f g h*.
- Group III may produce agglutinin *i k l*.

According to the American investigators referred to above (pp. 74—77) the pneumococcus, excluding the more or less distinct species *Pneumococcus* or *Streptococcus mucosus*, affords an example of such a species.

In such a method of grouping, as there is no overlapping of agglutinins, resort to the absorption test is not required.

But more commonly, particularly when a large number of strains is examined, the sub-grouping of a species is too complex a task to adapt itself to either of the simple schemes (a) and (b), and the question arises as to how far absorption is an aid to classification in these cases.

The complexity is due to the fact that the members of each proposed group are not identical in every respect. It is found, for example, that some proposed members of Groups I and II in scheme (b) produce, in addition to their special agglutinins *a b c*, *f g h*, some further agglutinin, *d* or *d e*, which is common to both groups, as in scheme (a). Again, it may be found that other members of a group, e.g. Group I in scheme (b), produce less than their special agglutinins *a b c*; some strains, as shown by absorption of the sera produced by them, may possess only the *a b* antigen, or only *a c*, or *b c*, or *a*, *b*, or *c*. Hence the absorption test, at first called in to justify the groups originally postulated, would make the further demand that these groups must again be subdivided.

Put schematically, the position would be:

(c) Members of Group I may combine with one or more or all of the agglutinins a, b, c, d, e .

Members of Group II, similarly, with d, e, f, g, h .

Members of Group III, similarly, with d, e, \dots, i, k, l .

Thus the agglutinin for the species as a whole would comprise all the components a to l , and each member would be identified by one or more of these components. If the individual members could be grouped as in the above scheme, *i.e.* sometimes with partial but never with complete overlapping of the main groups, grouping would be possible but the main groups would need further subdivision.

But here, as in the simpler case of the cholera vibrio, it is difficult to understand what diagnostic significance can be attached to a classification based on irregularities of absorptive capacity.

III. A SPECIES NOT DIVISIBLE INTO SUB-GROUPS.

It is not uncommon to meet with strains of bacteria possessing individual peculiarities which simply have to be recognised as such. This is no bar to classification, provided that the characteristic agglutinin for the species or for the sub-group remains demonstrable for every strain. But it is also possible that the members of a species may evince individuality of such a nature that they are not amenable to classification into groups.

As contrasted with Scheme (c), it would be impossible in this case to assign certain agglutinins as the exclusive property of one or other of the postulated main groups. Thus:

(d) The complete agglutinins of the species = $a, b, c, d, e, f, g, h, i, k, l$. Individual members may combine with only one, or more, or all of these.

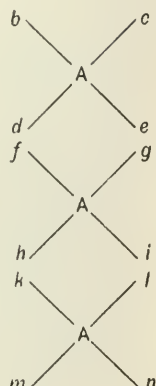
But the last scheme would not be a satisfactory representation of the species because it does not bring into expression the underlying unity of specific antigen upon which individual variations are superimposed. It would probably be more accurate to assume that specificity is based upon a common constitution of the protein molecule, to which a variable receptor apparatus is attached. Thus the agglutinin complex of each strain will possess a common characteristic, which may be designated A , and this, in the case of different strains, will be associated

irregularly with minor elements, *b, c, d, e*, etc., not individually representative of the species as a whole. For example:

(e) Strain (1) may produce agglutinin:—

„ (2) „ „ „

„ (3) „ „ „

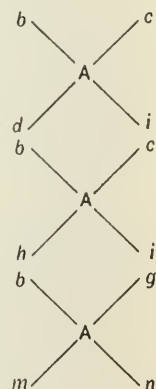


With these three strains *A* is shown as linked to four elements, and each of these is different for each strain. But other and less sharply contrasted expressions of individuality may occur, *e.g.*:

(f) Strain (4) may produce agglutinin:—

„ (5) „ „ „

„ (6) „ „ „



Strains (4), (5), (6) differ less from strain (1) than do strains (2) and (3); and it might be found that the agglutinin produced by strain (1), and therefore combining with strains identical with this, failed to combine with strains (2) and (3) but was able to unite with less divergent strains such as (4) and, though perhaps not equally well, with strains such as (5) and (6).

Similarly with agglutinin produced by strain (2). This might not affect strains (1) and (3) but it might interact with strains less divergent

from, though not identical with, the homologous strain. And the like would hold with strain (3) agglutinin.

Strains (1), (2) and (3) are taken as examples of strains sharply differing from each other, as shown by their respective elements $b c d e$, $f g h i$, $k l m n$. Obviously, the number of such strains may be very large: *e.g.* a fourth may be associated with $b f m g$, a fifth with $c h i k$, a sixth with $d e g l$, and so on. And, as illustrated above, each of these strains may produce agglutinin which will pick out not only strains identical with the homologous but also strains in which the difference from the homologous does not amount to a sharp contrast.

Hence, on testing the strains of this species with a large number of monovalent sera, the broad result would be a reiterated and irregular demonstration of resemblances and differences between different strains. The resemblances would be due to the fact that all the strains belong to the same species; the differences would be the expression of individual peculiarities. Scientific grouping would be impossible because, unlike the species discussed in Section II, the species now under consideration contains an indefinitely large number of strains which differ in qualitative capacity for producing and combining with agglutinin.

IV. THE QUESTION WHETHER A GIVEN SPECIES IS OR IS NOT DIVISIBLE INTO SUB-GROUPS.

It follows from the above considerations that if the strains belonging to the species fall into distinct groups, without cross-division, when tested with an extensive number of monovalent sera, grouping is indicated; but if such tests produce marked cross-division, grouping is not justifiable. For example, sera I, II and III may apparently divide meningococcal strains into three corresponding groups; but if sera prepared from other members of these groups invalidate this distinction, it may be inferred that the three groups do not represent an accurate subdivision of meningococcus antigen into three distinct and separable types.

V. GENERAL REMARKS.

In determining whether a doubtful organism belongs to one or other of certain different species, the absorption method is certainly useful sometimes, as a supplementary test, provided that the result is positive, *i.e.* that evidence of specific absorption is obtained.

The method has been applied clinically, by testing the patient's serum with bacteria of known species, and has been the means of iden-

tifying the bacterial cause of the disease, when the results of simple agglutination were ambiguous, and also of demonstrating cases of mixed infection. There are certain fallacies to guard against, as Paltauf has pointed out. The infection may really be due to an unsuspected organism, which has produced in the patient some heterologous agglutinin for one of the organisms under suspicion. For example, the diagnosis may be thought to lie between typhoid, paratyphoid, and Gärtner infection, and it may be found that the agglutinin which acts on these three organisms is absorbed by the typhoid bacillus; but the case may really be an infection with *B. proteus*, which has produced some heterologous agglutinin for the typhoid bacillus. But instances such as this do not detract from the fact that the method is admittedly useful. Similarly, when the organism isolated from the patient gives ambiguous agglutination results with laboratory sera representative of different species, a positive absorption test may help to settle the diagnosis.

On the other hand, a strain cannot be excluded from a species because the result of the absorption test is negative. Such cases, where the diagnosis must be determined by the general biological characters of the organism taken as a whole, serve as a useful reminder that serological reactions are not always infallible and do not necessarily play the decisive part in determining classification.

The sub-grouping of a species may turn out to be an easy or a difficult matter. This will depend partly on the homogeneity or the irregularity of specific antigen and partly on the use of a small or a large number of strains for the demonstration of agglutinogenic and combining capacities. It may be found, to begin with, that simple agglutination alone divides the strains into groups with no overlapping, as in Scheme (b). If the addition of more strains and more sera confirms this classification, well and good. If, however, it is now found that there is some overlapping, as in Scheme (a), resort will be made to absorption. This, possibly, will still support the original grouping, which will now be an amalgamation of Scheme (a) and Scheme (b). It may turn out, however, that in the enlarged series further differences of antigen are found between members of the same group, as in Scheme (c). Even now it may still be possible to provide a theoretical justification for the original groups, supplemented by a subdivision of each, provided that there is no more than a partial identity between the postulated complete antigen of each of the three main groups; though it hardly seems likely that such an elaborate classification would be of diagnostic value. At this stage, where each main group is so elastic that its margin of separation from the

others is small, one begins to raise the question whether the adopted system of grouping has not turned out to be artificial and arbitrary, and whether the species under consideration is really distinguishable from one which is not amenable to sub-grouping, as in Schemes (*d*), (*e*) and (*f*). One's decision will naturally be influenced by observing whether the proposed grouping does or does not involve the confusion of cross-division.

DIFFERENTIATION OF AGGLUTININS IN RELATION TO THEORIES OF IMMUNITY.

For the above discussion I have taken as the starting-point Durham's hypothesis of a multiplicity of agglutinins in monovalent immune sera, because his views are well known and have obtained wide, though not universal, acceptance. Like all other explanations of immunity which are based on Ehrlich's principles, Durham's theory postulates an indefinite or unlimited number of unknown chemical components, and on this ground it may be open to the objection, which has been raised against many of Ehrlich's postulates by the opposing school of Bordet, that this free coinage of hypothetical chemical entities is merely a re-statement of laboratory data in terms of the unknown, a resort, in fact, to the fallacious method of exposition known as *ignotum per ignotius*.

Perhaps there is some element of truth in this objection. For example, one might be tempted to begin by postulating that a particular agglutinin contained the components *a*, *b*, *c*; when laboratory facts came to light which showed that this explanation was insufficient, another component, *d*, might be tacked on to it; and this, when further occasion required, might be supplemented by *e*, *f*, *g*, etc. Obviously such postulates would be no real explanation but merely a redundant way of saying that the phenomena of agglutination are complex and of unknown nature.

At the same time one must recognise the importance of Ehrlich's general principle that the specificity of agglutinins is determined by their precise chemical constitutions, though these are too imperfectly understood to be expressed in the rational formulae of the organic chemist. This chemical conception, though unfortunately vague, cannot be ignored, because specificity cannot be explained on purely physical grounds; it must, however, be supplemented by the equally important conceptions of immunity which are based on experimental physics and the properties of colloids, since these principles, whilst not explaining specificity, play an essential part in agglutination.

The difficulty of Ehrlich's theory, it appears to me, is twofold; it is exclusively chemical and much weakened by controversial antagonism which refuses to recognise

the value of physical theories; and the chemical conceptions follow too closely analogies derived from the study of aniline dyes and experimental pharmacology. One needs a theory which will link up the chemical and the physical sides of the problem, instead of making them appear irreconcilable; and it is doubtful if the groups and side-chains which determine the properties of dyes and drugs are analogous to the differences in chemical structure which determine the differences of various antigens and antibodies. The fact that neither a purely chemical nor a purely physical theory will suffice is emphasised by Paltauf (1913), who has reviewed with remarkable impartiality conflicting theories as to the nature of agglutination¹. He concludes:—"Agglutination (and precipitation) is a genuine colloid reaction, but for the specificity of this reaction the chemical constitution of the interacting colloids is responsible. This determines the formation of the complex combinations which are associated with alteration of the conditions of solubility, and, by altering surface tension, determine the process of clumping."

The problem has been simplified by Bordet's conception, which has now been accepted by the majority of observers, that the agglutination reaction takes place in two phases, the first being "the period of impression" and the second "agglutination properly so called."

The result of the first phase is that a change takes place in the colloidal nature of the bacteria; this change involves, according to some physicists, with whom Paltauf appears inclined to agree, a conversion from the emuloid into the suspensoid form. Then, coming to the second phase, there is an interaction between two colloids (bacteria and serum) in the presence of salts, with consequent agglutination, if this is compatible with the physical condition of the bacterial colloid.

This view of Bordet's, now well established, serves to clear the ground and enables one to concentrate attention on the first phase, in which the specific interaction between antigen and antibody takes place.

Here chemical constitution must play a part, as it is difficult to see how physical conditions alone can determine specificity. The postulate of "specific absorption" merely postpones the difficulty, as the specificity of the interaction must depend on chemical constitution. Durham's conception of antigen and antibody as possessing a multiplicity of different chemical groups is at least chemical: and on this ground, it might be urged, it ought to be accepted until something better can be found to replace it. One way of emendation, advocated by some bacteriologists, is to introduce the conception of differences in "avidity" on the part of particular antigens. On this assumption, two conditions are necessary for the union between a particular antigen and a particular antibody, (1) appropriate chemical constitution and (2) the character termed "avidity." (1) is not necessarily accompanied by (2): when it

¹ Kolle and Wassermann's *Handbuch der pathogenen Mikroorganismen*, 2nd Ed., II. pt 1, pp. 483-654.

is not, union with antibody does not take place. The general conception of specific antigen and antibody as containing a large number of different chemical groups is retained.

I have already referred (p. 90) to the observations of Meinicke and his colleagues on differences in the absorptive capacities of cholera vibrios. Following Durham's hypothesis, one might endeavour to explain these results as in scheme (a) on p. 91, viz.

- Group I may contain antigens *a, b, c, d, e*.
- „ II may contain antigens *d, e, f, g, h*.
- „ III may contain antigens *d, e...i, k, l*.

The strains agree in agglutinability because they all possess the antigens *d, e*, but they differ in absorptive capacity owing to the presence or absence of certain other antigens; *e.g.*, absorption with a Group I strain will remove all agglutinin for members of this group, but not for strains containing any of the antigens *f...l*.

But against this explanation there are two objections. (1) The strains agree in agglutinability not merely with the same serum but with different sera; *e.g.*, though Group I and Group II do not absorb for each other, a Group I strain will produce a serum which agglutinates Group II strains and *vice versa*. (2) Under identical conditions of experiment, different strains absorb for themselves the same amount of agglutinin from different sera. (1) indicates that the different groups have not been found to differ qualitatively, *i.e.*, as regards the presence or absence of particular antigens; and (2) shows that they have not been found to differ quantitatively as regards the amount of particular antigens possessed, *i.e.*, it cannot be postulated that Group I strains possess all the antigens of the three groups (as shown by agglutinogenic capacity), but possess certain of these antigens in much smaller amounts than the other groups (as shown by lack of absorptive capacity); for if this were the case the amount of agglutinin which a strain absorbed for itself, under the same conditions of experiment, would differ according to the group-membership of the strain used for preparing the serum.

Meinicke and his colleagues meet this difficulty by postulating differences in "avidity." All their strains, they argue, possess all the cholera antigens, say *a* to *m*, but *in vitro* some of these elements lack "avidity" (capacity for combining with the corresponding agglutinin); thus, for one strain the only "avid" elements may be *a, b, d, k, m*; for another the elements, *c, e, f, n*, and so on. *In vivo*, however, the greater disintegration which takes place in the animal tissues releases all the antigens in an active condition, and consequently a serum is produced which contains every representative agglutinin.

This postulate of "differences in avidity" amongst different components of an antigen appears to me unnecessarily complex. I think the element of truth it may contain would be better expressed by a broad recognition of the fact that the combining capacities of antigen as a whole are affected by its particular chemico-physical condition.

Meinicke's experiments may then be regarded as showing that cholera antigen is one and the same, in essential chemical structure, and so is cholera antibody, but

minor differences exist *in vitro* and are brought out by the absorption test. This fact may be explained, not by representing a specific antigen as consisting of several different components coexisting side by side, but by regarding it as a chemical substance which may exist in one or other of several different chemico-physical phases, demonstrable by test-tube experiments. And the same conception would apply to antibody. Thus, when cholera culture and antiserum are brought together, the amount of culture being sufficient to remove the whole of the agglutinin with which it is capable of combining, the cholera antibody is affected as a whole by this interaction and any that is left uncombined settles down into equilibrium by a process of readjustment, involving such changes as constitute a new chemico-physical phase. The residual agglutinin, owing to its change of state, can only combine with such cholera strains as are in a different chemico-physical phase from the original absorbing strain.

This conception is not inconsistent with Paltauf's view that "according to the nature of the molecule as a whole, certain properties may vary, although the specific reacting group remains the same."

It is a conception which implies varying complexity in the structure of one and the same specific substance: it differs from the conceptions of Meinicke, Durham and others, which imply that specific substance is not one but multiple, and that each component is separable from the rest.

At this point it will be useful to give more definite significance to the term "chemico-physical phase" by reference to experimental facts. Apart from changes affecting only the second stage of the agglutination reaction, *i.e.*, changes in agglutinability without changes in absorptive capacity, variations have often been found in the absorptive capacity of the same strain under different conditions. Sometimes this change of condition is definitely due to a physical, chemical, or physiological influence and sometimes, when the reason of the change is unknown, one can only say that apparently spontaneous variations are found in nature.

As an example of physiological influence, artificially introduced, I may refer to the well-known fact that changes are often produced in bacteria by cultivation in immune serum. P. Th. Müller (1903)¹, for example, found that the agglutinability of typhoid bacilli was lowered by growth in immune serum and that this change was accompanied by a diminution in absorptive capacity. But this change, as Paltauf points out, does not always result from the action of immune serum. Some observers have found diminished agglutinability without diminished absorptive capacity; others have found no change in agglutinability; and others again have observed that strains cultivated in immune serum acquire the property of spontaneous agglutination. As another example of physiological influence, it may be mentioned that the characters of an immune serum often depend to an important extent upon the species of animal used for inoculation.

As regards purely physical influences, the effect of heat is the simplest example to take. The very extensive literature on this subject may be briefly summarised by

¹ *München med. Wochenschr.*, p. 13.

saying that exposure of a culture to a temperature above the normal may enhance, impair, or otherwise modify agglutinability, agglutinogenic capacity, and absorptive capacity.

Altman and Rauth (1910)¹ give a suggestive example of modification in serological properties produced by chemical means. With a particular strain of *B. coli* they produced a serum which, in agglutination and complement deviation tests, responded to the homologous strain alone. This strain, as shown by a month's passage on agar, remained stable and on separating out individual colonies it was found that all were serologically alike, being identical with the original strain. The strain was then treated by passage on carbol-agar, with the result that it lost its agglutinability. A serum was prepared from it and was found to agglutinate the carbol-strain but not the original strain. The properties of the new strain remained constant when the subcultures were made either on ordinary agar or on carbol-agar. Repeat experiments (three with one strain and one with another) produced similar serological modifications by passage on carbol-agar. During passage and before the change had been fully effected, a strain would react both with the ordinary serum and with the "carbol"-serum, and whilst in this transitional condition a strain would produce a serum agglutinating both kinds of culture. Three kinds of colonies could be obtained from such a strain, some reacting like the whole strain and others only with one or other of the two sera. The authors also found that somewhat similar changes could be produced in strains of *B. coli* by prolonged subculture in ordinary broth, the effect being that they lost their agglutinability with the original serum and sometimes became agglutinable with the "carbol"-serum. They suggest that the indol produced in the culture acted in the same way as phenol.

Bacteriological literature is full of examples of what may be termed natural irregularities or spontaneous variations. Perhaps two quotations will suffice for the purpose of illustrating this point.

Rufus Cole (1904)² illustrates differences in agglutinability of different strains of typhoid bacilli. He selected five laboratory strains, designated E, H, I, W and C, and ascertained their highest agglutinations with a particular serum. The results were: E = 8000; H = 7000; I = 4500; W = 4500; C = 4000. He then prepared a serum with I, one of the poorer agglutinators, and found: E = 3000; I = 700; C = 500. Then he prepared a serum with C and found it agglutinated E up to 3000 but only reached 2000 with C. Finally he compared the absorptive capacities of good and poor agglutinators on a serum which agglutinated E up to 5000. Four absorbing strains were used and reduced the titre for E as follows: E = 200; H = 500; W = 1000; C = 1000. Thus, as Cole points out, higher agglutinability was associated with greater binding capacity, and relatively poor agglutinability was a consistent feature of some of the strains even when these strains were used for the preparation of the serum. An interesting feature brought out by this short series of experiments is that the differences demonstrated between the good and the poor agglutinators are not such as to afford a basis for "serological grouping."

I have quoted this article because, although it only deals with a small amount of material, it exemplifies very well the experience of other observers on a large number of strains.

¹ *Zeitschr. f. Immunitätsforschung*, Orig. VII. 629.

² *Zeitschr. f. Hyg.*, XLVI.

Solernheim and Seligmann (1910)¹ call attention to biological variations in strains of *B. paratyphosus* (B) and *Gaertner*. They point out that if a laboratory only uses one standard serum for each of these groups of organisms, new strains can generally be accounted for, though with some exceptions. But the results are much more complicated if a large number of strains are tested simultaneously with a large number of sera. Their observations are based on examination of 100 paratyphoid and Gaertner strains and 60 sera.

As regards the Gaertner group, they found that a high titre serum agglutinated only a certain number of strains: others were slightly affected, and many others were left untouched. Comparing the individual results with different sera, further differences came to light; the sera failed to tally either as regards degree of agglutination produced or as regards number of strains agglutinated; hardly any of them affected all their strains. Some strains showed changes in course of time in their agglutinability and agglutinogenic properties; they would pass from good into poor agglutinators or the reverse change would occur, and transitional forms were met with. They had two strains which, for a time, were not affected by any Gaertner or paratyphoid serum and produced a serum which agglutinated themselves alone. But these were *bona fide* Gaertner strains to begin with and subsequently reverted to this type, agglutinating with Gaertner serum up to full titre. Then, when the cultures were plated out and examination was made of separate colonies, agglutinable, in-agglutinable, and intermediate colonies were found; and cultures from these exhibited corresponding agglutinogenic differences.

Irregularities and variations were also found in some of the paratyphoid (B) strains. Six strains were typical to begin with but gradually changed in agglutinability. They were then plated out and two kinds of colonies were found, (1) round, translucent colonies which agglutinated like paratyphoid (B), and (2) colonies with granular surface and irregular margin which agglutinated with both paratyphoid and Gaertner serum. Colonies of the second type were plated out four times to confirm their purity, always with the same result; they produced pure paratyphoid (B) serum which had not a trace of influence on Gaertner strains. From another strain, originally an ordinary paratyphoid (B), the daughter cultures were found to be of much lower agglutinogenic power. The serum produced was a pure paratyphoid (B) serum but it only acted on some of the paratyphoid (B) strains and on these, for the most part, not completely; on the other hand, it agglutinated strongly and to high titre not only all the strains which reacted to both paratyphoid and Gaertner sera but also those which had been found to give hardly any reaction with other paratyphoid sera. Finally, from a paratyphoid (B) culture they separated out a strain which was agglutinated by Gaertner but not by paratyphoid sera; but it produced a serum of paratyphoid (B) character, with marked preference for strains in the transitional stage. As the authors remark, the above results show that capacity for binding agglutinin is not necessarily parallel with capacity for producing agglutinin.

The view that certain variations in the combining capacities of antigen and antibody may be attributable to changes in the "chemico-physical phase" of one and the same specific substance leads one to

¹ *Deutsche med. Wochenschr.*, p. 351

consider the possible influence of minute variations in stereo-chemical structure.

A striking feature about the chemistry of bacteria is that these organisms have a remarkably selective action upon sugars and other allied compounds which are closely related to each other and differ only, or mainly, in stereo-chemical configuration. This selective action, perhaps comparable to the selective action of certain alkaloids, such as brucine, upon sugars which differ only in stereo-chemical respects, may indicate that in the molecules of the bacterial protoplasm there are groupings, linked to asymmetric carbon atoms, which act as "receptors" for the corresponding groupings linked to the asymmetric carbon atoms in the sugar molecules.

This direct evidence of the importance of stereo-chemical structure suggests that differences and affinities of a stereo-chemical nature may also play an important part in the constitution of antigen and antibody and in the relationship of the one to the other.

In this connection one must refer back to the views expressed by Emil Fischer. Writing in 1898 on "The Significance of Stereo-chemistry for Physiology"¹ he has developed the theory, which he had foreshadowed in 1894, that the selective action of enzymes depends on their asymmetric structure. Though the nature of enzymes is not definitely known, because they have not been isolated as chemically pure compounds, "yet their resemblance to proteins is so great and their origin from the latter is so probable that they must undoubtedly be regarded as composed of molecules which are optically active and asymmetrical. This," he continues, "has led to the hypothesis that between enzyme and fermentable substance a similarity of molecular configuration must exist, if a reaction is to follow. To make this idea clearer I have used the metaphor of lock and key." He is far from regarding this hypothesis as an established scientific theory, and admits that it cannot be fully substantiated until enzymes are isolated in a pure state and their configuration is investigated; but he regards it as a fruitful hypothesis and has found it helpful in the orientation of chemical research.

Fischer's conception is based on his study of the sugars and allied compounds, which has provided extensive corroboration of the principles of stereo-chemistry founded by Pasteur, Le Bel and van't Hoff. Whether the same conception is capable of useful application to immunity problems is another matter; but it is at least worth considering. From the stereo-chemical standpoint, in so far as it may concern immunity problems, some of the salient facts which have been demonstrated by research on pure compounds of known chemical constitution are:

¹ *Zeitschr. f. physiolog. Chemie*, xxvi, 60.

(1) A pure compound may be produced not only in the optically active forms *d* and *l* but also in forms which are optically inactive. In the case of the latter, inactivation or compensation may take place either externally, *i.e.*, by union of a *d* molecule with an *l* molecule, or internally, *i.e.*, by compensation within the molecule of a *d* group and the corresponding *l* group. A compound inactive by external compensation can be split up into equal numbers of *d* and *l* molecules; but when the compensation is internal, similar dissociation is impossible, as the separation of the *d* and *l* groups would involve the disintegration of the molecule. The four tartaric acids are usually quoted as the classical examples of these facts.

(2) Enzymes often exhibit a selective action upon the *d* and *l* forms of the same optically active compound, fermenting the one but leaving the other unaltered; and their action upon compensated forms will depend upon whether these can be dissociated into active forms.

(3) Pure compounds are found which are almost identical in chemical structure, the only differences being a stereo-chemical difference in the position of groups linked to an asymmetric carbon atom, *e.g.*, the "right-handed" or "left-handed" position of the groups —OH and —H. The best known examples of these facts are found in the sugars of the 6th series.

(4) Enzymes have a selective action on substances differing only in the slight degree mentioned in (3).

There is, I think, reasonable ground for expecting that, when the chemistry of proteins is better known, stereo-chemical conceptions will correct and greatly simplify Ehrlich's very elaborate but highly artificial theory of an indefinite multitude of "side-chains." From the bacteriological side, this line of explanation seems indicated, perhaps most definitely, by the irregularities and the apparently anomalous results such as are often met with in agglutination reactions. Such apparent discrepancies, when irreconcilable with the working hypothesis which the investigator finds suitable to the majority of his data, are sometimes dismissed as negligible. A serum which only agglutinates a few of the strains it was expected to agglutinate is "not useful"; a culture which does not agglutinate with its assumed "standard" serum is "in poor condition"; if in later subculture it behaves as had been expected previously, it has "come up to standard"; if it agglutinates well at first but falls off subsequently, it has "deteriorated"; at all events these little incidents or accidents "don't count."

I admit it would be difficult to account for them by a Jack-in-the-box appearance, disappearance, or neutralisation of fixed chemical groups or side chains constituting the postulated collection of antigens and antibodies; and I think the fact of their occurrence suggests a modification of this conception in favour of the view that, in the interaction of compounds containing asymmetric carbon atoms, many minor

changes of a stereo-chemical nature will occur (*i.e.* changes not involving elimination or introduction of fixed chemical groups), and that these minor changes may often suffice to produce very striking differences in serological reactions.

Some, at least, of the irregularities which have been demonstrated in the agglutination reactions of particular species of bacteria may perhaps be attributed to stereo-chemical differences of one and the same specific substance rather than to the production of an indefinite variety of different chemical components; and these differences may concern agglutinability and agglutinogenic capacity as well as absorptive capacity. Thus there are the differences due to: (1) *Storage*: when tested with the same serum, a freshly prepared culture emulsion may differ in agglutinability from the same emulsion tested after keeping for some time; (2) *Heat*: a heated culture emulsion may differ in agglutinability and agglutinogenic capacity from the same culture unheated; (3) *Subculture*: earlier and later subcultures of the same strain may differ in agglutinability and in agglutinogenic capacity; (4) *Conditions of growth*: changes may be produced by environment in the animal body or by the nature of the medium used for culture. And the possibility of similar non-specific differences must be considered when comparing one strain with another.

In the last paragraph I have been considering conditions affecting antigens. Possibly analogous differences may exist in the stereo-chemical condition of the specific antibodies to a given organism. Such differences may be due to: (5) *The animal body*: when animals of different species, *e.g.*, the rabbit and the horse, are inoculated with the same strain, it is found that some of the sera are more multivalent than others, and similar differences are sometimes found between sera from animals of the same species; (6) *Condition of culture used for immunising*: the sera produced may vary according to the condition of the culture as regards (1), (2), (3) and (4); (7) *Storage of serum*: a serum may deteriorate on keeping. Again, the non-specific conditions (5), (6) and (7) must be considered when comparing the specificity of different sera.

Furthermore, in the reaction between antigen and antibody, varying stereo-chemical conditions of the two substances may suffice to explain some irregularities in tests for absorption of agglutinin. When the optically active part of a simple organic compound is inactivated in its behaviour towards polarised light by the presence of its geometrical counterpart, this inactivation may be (*a*) either complete or partial and (*b*) either readily annulled (external compensation) or firmly fixed (internal compensation). Similarly, the union of agglutinin with antigen may be (*a*) either complete or partial and (*b*) may be firmly fixed or may lead with greater or less readiness to a stereo-chemical re-arrangement.

This stereo-chemical view of the conditions which play a part in determining the chemico-physical and specifically chemical interaction between antigen and antibody in the first phase of the agglutination reaction leads to a comparison between the interaction of antigen with antibody and the interaction of a ferment with a fermentable substance. In the latter reaction, the feature which is commonly conspicuous is

that union between the two substances and consequent chemical alteration of the one (the fermentable substance) is followed by complete, or almost complete, dissociation, leaving the ferment free to act upon more fermentable substance; thus a small amount of ferment may, so long as the medium remains favourable for the reaction, and until a condition of equilibrium has been established, act upon an indefinitely large amount of fermentable substance. In the case of antigen and antibody conditions are different in one important respect, in that antigen is unable to combine with or modify an indefinitely large quantity of antibody, and the amount of combination effected depends, *ceteris paribus*, on the amount of antigen present. When the absorbing strain is used in sufficient amount, it renders the serum incapable of agglutinating a further supply of the same strain, or of other strains which are identical in every chemico-physical detail, just as a medium upon which an enzyme has exerted its full effect will not be influenced by the introduction of a fresh supply of the same enzyme. It will be noted that in making this comparison I think it advantageous to regard the enzyme as comparable to the antigen rather than to the antibody.

This result, the removal of a certain agglutinating capacity from the serum, must involve a chemical interaction taking place in the first stage of the agglutination reaction, because cultures which are devoid of agglutinability, either naturally or as a result of experimental treatment, often retain their capacity for "binding agglutinin."

Taking chemical considerations first and postponing the question of physical influences, the usual, and generally the most convenient, way of expressing the experimental facts is to say that the culture removes agglutinin from the serum. This may be true, but it does not follow that it is accurate to regard antibody as a mixed collection of assorted goods from which a culture removes a greater or smaller number of articles and leaves the rest as they were before the reaction took place. The interaction, when absorption takes place with excess of culture, should probably be regarded not as a simple subtraction of certain fractional parts of antibody but as involving partly a combination with antibody and partly a modification of the residuum, and resulting in a new phase of equilibrium which allows antigen to persist in the uncombined state when in the presence of the modified antibody which remains.

This modification of antibody occasionally manifests itself in a paradoxical manner, when it is found that a serum absorbed with a particular strain gives a higher titre for some other strain than it did before absorption. Obviously, in this case absorption has not been a process of simple subtraction of agglutinin, and it is

not very helpful to call it a subtraction of an inhibitory influence; it seems rather a change of "chemico-physical phase" in the direction of increased activity; and in other cases, where the titre of the absorbed serum is unaltered for some strains and greatly or slightly diminished for others, the changed state of residual antibody is an experimental fact but the postulate that some fractions of antibody have been removed and others left "just as they were" seems too crude to be likely to be true.

Fischer's "lock and key" metaphor, as employed by him in a strictly stereo-chemical sense, certainly seems helpful in the interpretation of immunity reactions, if applied in the right place. In the first stage of agglutination the union of antigen and antibody may be regarded as determined by their stereo-chemical configuration, like the union of enzyme and fermentable substance, though antigen is incapable of uniting with an indefinitely large quantity of antibody, because union is not followed by simple dissociation (catalytic action). The union appears to be associated with the production of stereo-chemical changes in the residual antibody which render impossible any further union with the particular stereo-chemical type of antigen employed.

At the same time it must not be assumed that this conception will explain everything. There are not only qualitative, or stereo-chemical, but also quantitative differences of absorptive capacity, the latter probably of a colloidal or physical rather than of a chemical nature. Here it is difficult, if not impossible, to draw a sharp distinction between the first and the second stage of the agglutination reaction. When the reaction has been completed, three facts usually stand out prominently. (1) For the production of agglutination some strains require more agglutinin than others, as shown by differences in titre. (2) The amount of agglutinin removed in "saturation" experiments is more than enough to have agglutinated the whole of the culture used, *i.e.* a much smaller quantity of serum would have been sufficient for agglutination. (3) The quantity of culture required to "absorb" the same amount of agglutinin often differs with different strains.

These facts suggest physical as well as chemical influence. As the reaction is a lengthy process, as compared with the prompt interaction of simple chemical compounds, it may be considered that the "period of impression," as well as "agglutination proper," requires considerable time for its completion, though it may commence immediately, and also that the physical factors characteristic of the second stage begin to exert their influence before the process of chemical union, characteristic of the first stage, has come to an end. In other words, the "period of

impression" may be associated with physical absorption as well as with chemical interaction.

Hence differentiation by absorption is not a purely qualitative criterion in the chemical sense, because the results obtained are partly determined by quantitative or physical conditions. In so far as the latter can be disregarded, the more strictly chemical conditions emerge more clearly, viz.: (1) the presence of antigen and antibody which are *specific*, in that they are of definite chemical constitution, but may exist in one or other of several different geometrical shapes; (2) the *special* stereo-chemical condition of antigen and antibody which determines whether, "like lock and key," they can approach each other closely enough for the production of a chemical reaction.

If it be possible, as suggested in the preceding discussion, to reconcile the fundamental principles of Ehrlich and Bordet by the connecting link of stereo-chemistry, conceptions of classification by serological reactions will need revision.

Owing to the extreme complexity of the chemical substances concerned and the lack of accurate knowledge as to their nature, this is a problem which is very far from solution; but, though direct chemical analysis of antigen is at present impossible, some advance in this direction may be made on the bacteriological side by analysis of the serological effects attributable to those variations in the structure of antigen which are probably of a stereo-chemical nature.

Detailed study of agglutination, absorption of agglutinin, and agglutinogenic capacity has already proved of value by showing the complexity and variability of serological reactions amongst different members of one species, particularly when many strains and many sera are used and compared. Some strains exhibit one phase of this complexity and some another; and so strains may be sorted out into a considerable number of groups (overlapping to greater or less extent) according to the phase which each exhibits. The number of groups will depend on the number of strains investigated and on the range of serological tests employed; it will increase from time to time as the investigation assumes wider dimensions. Meinicke remarked about the grouping of his cholera vibrios by absorption tests that such a classification is of no practical utility. But the demonstration that such serological differences are forthcoming serves two useful purposes: (1) it shows the unity, underlying minor differences, which characterises members of a species; and (2) it shows that the erection of these minor differences into class distinctions, attributable to the presence of distinct and separable antigens,

would lead to such a large and confusing subdivision as to invalidate the hypothesis on which it was based.

As Uhlenhuth and others have remarked, confusion may not arise if attention is limited to a small number of stock laboratory strains and a few sera; some of these strains might absorb homologous agglutinin from one serum and the rest might absorb it from a second serum, and so no more than two groups would need to be postulated. The confusion comes into prominence when a large and unselected series of freshly isolated strains of a species are fully tested in all their serological capacities.

It must also be borne in mind that serological differences brought out by agglutination tests, though of minor importance in some respects, may be associated with differences of an antitoxic or antibacterial nature, and so may possibly give a clue to the selection of sera for therapeutic purposes.

It is generally agreed that for some organisms, such as the cholera vibrio, antibody is of a simpler nature than is the case with many other species of bacteria wherein strains differ from one another in both agglutinability and agglutinogenic capacity. Here again it may be possible to borrow an analogy from organic chemistry. If the simpler antibody be compared to a sugar of the sixth series, say glucose or galactose, the more complex antibody may perhaps be compared to one of the higher sugars, say lactose, which can be split up into simpler sugars (in this case into glucose and galactose). The species with the complex antigen may produce corresponding agglutinins which reveal a strong indication of division into two groups, though closer enquiry may show that these groups are not sharply separable, and that some strains possess characteristics of both groups. That is what might be expected from a "lactose" antigen or antibody which, under varying conditions affecting its stability, might present resemblances sometimes to glucose, sometimes to galactose, and sometimes to both. That might explain why a strain might not be consistently "glucose" (or "galactose") in both agglutinability and agglutinogenic capacity.

As regards differences between agglutinability and absorptive capacity (agglutination with a certain serum but failure to remove agglutinin for the strain producing the serum), the explanation might be referred, as with the less complex cholera vibrio, to stereo-chemical variation.

PRACTICAL SIGNIFICANCE.

These theoretical questions have a direct bearing on diagnosis by agglutination tests, particularly concerning the application of Castellani's method to problems other than those of mixed infection.

In a mixed infection, *e.g.* one produced by two organisms, A and B, of undoubtedly different species, everyone would concede that two different antigens are concerned, with correspondingly different antibodies, and that Castellani's method of separating them out is often useful, particularly when there happens to be some accidental or at least non-specific interaction between A antigen and B antibody. In such a case there is no urgent need to formulate any particular theory as to the nature of the reaction. Admittedly, antigen is a very complex substance, and so is antibody; the question whether the interaction takes place between A as a whole and B as a whole (with resultant changes in residual A and B) or merely between one separate non-specific component of A and another similar component of B (residual A and B being unaffected) need not be answered, because either alternative would be in accordance with Castellani's results.

But with two strains known to be of the same species, *e.g.* two cholera strains, wherein absorption tests revealed differences between the two antigens, one's theoretical conception of the interaction between antigen and antibody must be substantiated. Here there is no justification for the assumption that the differences are due to the presence of a non-specific component in the antigen of each strain. Such an assumption would soon be found to be untenable if applied to a large number of strains of this species; so many components of antigen would then turn out to be non-specific that ultimately no demonstrable components of specific antigen would be left.

Still more necessary is it to challenge underlying theoretical assumptions as to non-specific components in dealing with a case presented for diagnosis, *i.e.* in answering the question whether an unknown organism can be proved to be of different species from a known organism by the adoption of the Castellani method. If this question is seriously *sub judice*, one must give the organism in question the benefit of a fair trial, and therefore one must recognise the validity of the plea that the unknown and the known organism may be of the same species though differing, like cholera vibrios, in absorptive capacity.

Hence, in dealing with organisms which may possibly be of the same species, in virtue of their morphological and cultural identity, one cannot accept any deductions from theoretical considerations of immunity which are so framed as to permit an arbitrary sorting out of the agglutinins produced by such organisms into specific and non-specific components.

OBSERVATIONS BY DRS GRIFFITH AND SCOTT.

I will now bring my discussion of the absorption test into relation with the observations made by Dr Griffith and Dr Scott.

Dr Griffith has shown that simple agglutination tests suffice to effect a rough division of meningococci into two main groups, provided that carefully selected sera are used; but this division cannot be strictly maintained with all sera, because the grouping produced by some would be different from that produced by others. His explanation is that the grouping, being based on a response to the predominant agglutinins contained in the serum, is determined by the antigen used to produce these; but strains, though alike in agglutinability with the selected "group" sera, may differ from each other in the properties of their antigens and hence may produce different agglutinins. This rough grouping has, however, been found very useful for orientation purposes, before proceeding to a more precise analysis of antigen. In comparing cerebro-spinal with naso-pharyngeal strains, it has brought into prominence the fact that, whereas the two collections of strains are about equally represented in Group II, representatives of Group I are common in the former collection but rare in the latter.

If the system of grouping were infallible, one might infer that Group I antigen is rarely present in the naso-pharyngeal meningococcus of the non-contact. But as the system is far from being perfect, a second alternative has to be considered; the naso-pharyngeal meningococcus may contain Group I antigen and its presence may be demonstrated by the use of other sera prepared from cerebro-spinal strains containing that antigen. Three such sera have been made by Dr Griffith and have been found to agglutinate several naso-pharyngeal strains strongly enough to indicate the presence of Group I antigen in these.

This last observation furnishes a clue to the analysis of antigen which Dr Griffith has followed up in his study of agglutinogenic capacity. Sera were prepared from six naso-pharyngeal strains, none of which could be identified with Group I by simple agglutination tests. The sera, however, gave good agglutination with several cerebro-spinal strains in both groups and exhibited a more uniform influence on those belonging to Group I; they generally failed to agglutinate the naso-pharyngeal strains which were agglutinated by the standard Group II serum, but were fairly consistent in agglutinating those naso-pharyngeal strains which were not affected by this serum. Tests for agglutinogenic capacity, therefore, show that certain naso-pharyngeal strains possess

both Group I and Group II antigens. Similar characters have been demonstrated for a few of the cerebro-spinal strains which could not be classified by simple agglutination as either Group I or Group II.

Dr Griffith's next step in the analysis of antigen was to resort to the absorption test, which, he finds, gives more precise information than simple agglutination as to the combination of agglutinin and antigen and defines more clearly than the agglutinogenic test the degree of relationship between the antigens of different strains. Taking 22 spinal strains of Group I and testing them with six sera, he has found that they differ in range of absorptive capacity. Some strains remove from the sera all the agglutinins demonstrable, which he designates A, B, and C; other strains remove C only, others B and C, and others A and C. These results he has confirmed by increasing the quantities of culture used for absorption: as the differences remained unaffected, he concludes that they are qualitative and not quantitative in character. Corresponding to these differences he postulates three components, A, B, and C, in the antigen of Group I strains. From a similar analysis of Group II spinal strains he has shown that there are at least four different Group II agglutinins.

On comparing naso-pharyngeal and cerebro-spinal strains as regards combining capacity, he finds that those of the former origin which agglutinate well with Group II spinal sera also exhaust the homologous agglutinin of one or more of the four representative sera.

Of the remaining naso-pharyngeal strains, a few, which were agglutinated by Group I sera, have been found to absorb one or more of the three Group I agglutinins.

There is, however, a larger residue of naso-pharyngeal strains which could not be classed by agglutinability as either Group I or Group II. By means of the absorption method, taken in conjunction with agglutinogenic tests, Dr Griffith has shown that several of these possess components of both Group I and Group II antigen. In this respect they resemble two spinal strains which also failed to agglutinate distinctively with Group I and Group II sera and were unable to absorb any of the three components of Group I agglutinin.

The observations which I have briefly summarised in the preceding paragraphs enable Dr Griffith to expand his conception of the nature of meningococcus antigen. He regards it as a substance which all meningococci possess in common, irrespective of their origin and irrespective of their classification as Group I, or Group II, or indeterminate. But it is a complex substance and different strains manifest its com-

plexity in different degrees and in different ways. In some strains, which cannot be grouped either as I or II, he regards antigen as being in its least complex phase, with the Group I and the Group II elements about equally balanced. In others complexity is increased by a preponderance of one, two, or all three of the Group I elements designated A, B and C. In others, again, there is a similar preponderance of one or more of the elements characteristic of Group II. Underlying these differences, however, there is the same specific substance possessed by all strains in common; and it is the presence of this which explains why strains which differ markedly in agglutinability can be shown to be inter-related by agglutinogenic and absorption tests.

In support of this view Dr Griffith calls attention to modifications in antigenic capacity which some of his strains have exhibited in the course of sub-culture. These changes, he considers, can be explained as modifications of a primary antigenic substance in one or other of two directions, involving increase or diminution of complexity. A similar conception, he holds, would explain changes in antigenic characters which may be attributable to the influence of the human tissues and are characterised in cases of cerebro-spinal fever by the acquired capacity of invading the meninges, a capacity which appears to be much more capable of development in strains possessing the Group I type of antigen than in strains with the less complex antigens which cannot be relegated either to Group I or Group II. These modifications of antigen may, he suggests, run parallel with the curve charting the course of an epidemic, increased complexity of antigen being associated with the upward curve and decreased complexity with the downward curve.

In commenting on Dr Griffith's observations I think it will be useful to call attention to the differences in mental attitude which bacteriologists have adopted towards the absorption test. Some observers, impressed by the fact that this test is often a valuable aid to diagnosis, emphasise, and perhaps over-emphasise, its value as a bacteriological criterion. On the other hand, there is a school of bacteriologists who emphasise, perhaps unduly, the marked differences in absorptive capacity which may be exhibited by strains undoubtedly of the same species; hence they are disposed to minimise the value of the method for diagnostic purposes. I think Dr Griffith's work will help to bring about a reconciliation between these opposing views. He has shown that the absorption method is of great value in throwing light upon the structure of antigen; but, as the structure is complex and liable to variation, great care is requisite in the interpretation of absorption results. On

the other hand, he does not find that absorptive capacities are so irregular as to be unsuitable for scientific analysis; he shows that their variations appear to be determined by definite principles, and that they are capable of a classification which is systematic though as yet incomplete. He considers that the variations are dependent on minute changes of structure; these, I have suggested, may be largely determined by conditions of a stereo-chemical nature. I agree with his view that the changes can be explained as modifications of a primary antigenic substance; and I think this explanation is preferable to postulating the introduction from without of an antigen originally alien to the strain, or the removal from within of an antigen which the strain originally possessed.

Dr Scott has made an independent investigation of the same problem, using a different set of strains from those employed by Dr Griffith. Like Dr Griffith, he has found that his strains can be roughly divided into two main groups and that the cerebro-spinal strains are well represented in both groups, whilst the naso-pharyngeal strains preponderate in Group II, but are conspicuously rare in Group I. On minute analysis he finds that this rough subdivision does not suffice for a complete classification of the strains he has examined. Simple agglutination reactions alone afforded an indication that, in addition to the two main groups, there were at least five smaller groups more or less related to Group I and at least two small groups related to Group II. This further subdivision he has confirmed and rendered more precise by the application of tests for the absorption of agglutinin. But the adoption of this classification for practical purposes was found to be confronted by two difficulties; variations in agglutinability and absorptive capacity were so great as to make the classification uncertain, and some strains were found, both spinal and pharyngeal, which could not be placed as serological members of any of the groups. Hence Dr Scott concludes that it is impossible to regard his types or groups as representing distinct classes limited by hard-and-fast lines.

I agree with this last conclusion of Dr Scott's. In other respects I regard his results as being confirmatory of Dr Griffith's in their bearing on the diagnostic significance of serological reactions. Where the results of the two observers do not tally exactly, the differences are probably attributable to the use of different sera. For example, Dr Scott does not appear to have obtained any sera from Group I strains presenting the high degree of antigenic complexity exhibited by some of Dr Griffith's Group I strains.

CONCLUSIONS.

In my introduction to this report I raised the question whether serological tests could be found which would differentiate cerebro-spinal meningococci from naso-pharyngeal meningococci carried by non-contacts. This question opens out a wide problem, which I have presented as a series of definite issues, following one after the other, and each demanding a practical solution. Reverting to the order in which I set them out, I think the following answers may be returned to the questions raised as to the value of serological tests for the diagnosis of the meningococcus.

(1) How many standard sera would be required? In replying to this question one must first raise objection to the term "standard." Serologically, the meningococcus is unlike such bacteria as the typhoid bacillus and the cholera vibrio which, on the whole, are uniformly good agglutinators and therefore may be expected to conform to a serological standard. The meningococcus is one of many organisms which are much less constant in their response to agglutination tests, no matter what serum is employed, and therefore do not necessarily conform to any serological standard. The more appropriate question would be: How many sera would be required to form a useful aid to the diagnosis of the meningococcus? The answer is that two sera would suffice for the greater number of strains, provided that the one was a typical Group I and the other a typical Group II serum. There would remain some strains which were not hit off by either serum. If sera were produced from some of these, the number of strains not found amenable to the agglutination test would be diminished, but one cannot say more than that; it would be quite arbitrary and unjustifiable to fix a numerical limit of three, four, or any greater number of sera and to claim that every meningococcus must agglutinate with one or other of these.

(2) Would there be identity of standards in different laboratories? Every laboratory would have a Group I and a Group II serum, but probably the two sera employed in different laboratories would not be identical, as the range of activity of these sera depends to a very important extent on the particular strains used for immunisation. As regards subsidiary sera there would be still less likelihood of identity.

(3) Would simple agglutination tests necessarily be diagnostic? Irrespective of cultural tests, agglutination is not sufficient, because other organisms, *e.g.* the gonococcus, may agglutinate with a meningococcus serum. With organisms corresponding to the meningococcus in

all other laboratory tests, a positive agglutination result is confirmatory, but a negative result, even with several sera, is not decisive.

(4) What is the value of agglutination when supplemented by tests for absorption of agglutinin? A positive result of the absorption test is confirmatory, but absorptive capacities are too irregular to justify any diagnostic significance from negative results.

(5) Is the absorption method valid? The absorption test proves nothing when the result is negative, *i.e.* negative results do not disprove membership of a species, as indicated by other biological characters.

(6) What is the value of sera prepared with naso-pharyngeal strains from non-contacts? When not identical with sera obtained from cerebro-spinal strains, they demonstrate inter-relationship between cerebro-spinal strains and such naso-pharyngeal strains as are not agglutinated by the cerebro-spinal sera available. They show no indications of any serological characters common to naso-pharyngeal strains and distinguishing these from strains of cerebro-spinal origin.

(7) How do theoretical considerations of immunity affect the practical problem of diagnosis? They show that Castellani's principles of differentiation by absorption of agglutinin, taken in conjunction with Durham's postulate of multiple components of antigen and antibody, cannot be regarded as an infallible criterion for the identification of species.

(8) Are serological tests necessary before deciding whether an organism is or is not a meningococcus, *i.e.* capable or incapable, under favourable circumstances, of producing cerebro-spinal fever? No: cultural tests, if adequately performed, will suffice.

APPENDIX.

THE RELATION OF THE BOARD'S BACTERIOLOGICAL INVESTIGATIONS TO OTHER RECENT ENQUIRIES ON MENINGOCOCCUS CARRIERS.

In my last report I presented a historical survey of the literature up to the end of 1914, *i.e.* up to the period immediately preceding the epidemic of cerebro-spinal fever in this country. This outbreak has led to a large number of investigations throughout the country, dealing chiefly with the meningococcus problem as it has affected the military forces.

The bacteriological reports on this work, for the year 1915, have been reviewed by a Special Advisory Committee which reported to the Medical Research Committee in 1916¹. The Committee states (p. 32)

¹ *Medical Research Committee. Special Report Series. No. 2.*

that "a very large part of the work of most of the reporters has lain in the routine examination of the pharynx of contacts with cases of cerebro-spinal fever." It continues (p. 33):—"In contrast to their experiences with the cerebro-spinal fluid, the great majority of the reporters complain of the unsatisfactory results of the methods for determining the presence of the meningococcus in the naso-pharynx....The work is tedious and beset with pitfalls, while its results were often found ambiguous. Two of the reporters, indeed, express a doubt whether the swabbing of contacts is of sufficient value to be worth the trouble involved." On the use of agglutination as a test for the meningococcus, the Committee says (p. 14):—"Some of the reporters have tried this mode of diagnosis. The best methods for its employment were determined some years ago, chiefly by the German workers....Most of those who have employed this test seem to have found it capricious and unreliable." And again (p. 33):—"In the present series of reports agglutination has been little used as a confirmatory test, or tried and found too unreliable to be of service. Major Gordon now believes that the methods and special sera which have been introduced at the Millbank laboratories will in future form the most speedy and reliable confirmatory tests available."

Subsequent to the issue of this Committee's report, the work of Gordon and his associates has been brought up to date in a special volume, published in 1917¹. The relationship of this work to that conducted in the Board's Laboratory calls for some notice.

The laboratory work done for the military authorities was organised with a view to dealing with a special emergency. Cases of cerebro-spinal fever had occurred amongst the troops and, with the object of preventing the spread of the disease, it was decided to swab contacts and isolate all men found to be carriers of cocci which might be regarded as dangerous. The dangerous cocci were eventually defined as those which were found by every available test, serological as well as cultural, to be identical with meningococci isolated from recent cases of cerebro-spinal fever. Of the contacts examined, those found to be carriers of such cocci were to be reported as "positive," the others as "negative." To facilitate prompt diagnosis, a routine procedure of laboratory tests was laid down, and special culture media were provided, together with certain varieties of sera prepared from strains regarded as representative of the different varieties of meningococci discovered in the epidemic then prevailing.

¹ *Medical Research Committee. Special Report Series. No. 3.*

The work in the Board's Laboratory was arranged with a different object in view. Routine work on the meningococcus was limited to the diagnosis of specimens of cerebro-spinal fluid sent by medical officers of health in England and Wales, whilst the carrier question was treated entirely as a research problem, involving enquiry, irrespective of previous bacteriological findings or provisionally accepted opinions, as to the presence or absence of meningococci in the naso-pharynx of non-contacts. Hence there is very little basis for comparison between the results obtained in the Board's Laboratory and the laboratory data furnished to the military authorities.

The investigation of the non-contacts at St Bartholomew's Hospital will serve as an illustration. When Dr Griffith and I first found that a considerable number of these patients yielded strains which were culturally indistinguishable from meningococci, we refrained from making a "positive" diagnosis for six months or more, because we considered that the serological reactions of the strains should be fully worked out before arriving at a decision. Again, when certain of these strains failed to agglutinate with sera prepared from cerebro-spinal strains, we did not regard this result as decisive in favour of a "negative" diagnosis, because sometimes meningococci from cases of cerebro-spinal fever, like pneumococci from cases of lobar pneumonia, may fail to agglutinate with any serum prepared from so-called "standard" strains. In short, it was not our business to follow a prescribed schedule of tests which would determine automatically for each strain whether it was to be reported as "positive" or "negative"; the task was to make full investigation of the individual idiosyncrasies of both cerebro-spinal and naso-pharyngeal strains: and it was only when this work had been in progress for about a year that it was decided that the latter strains must be regarded as true meningococci. This delay caused no inconvenience, because no restrictions of any sort were contemplated for the carriers discovered.

I have no hesitation in saying that, if we had been required to make prompt diagnosis according to the schedule of procedure laid down by the military authorities, the "negative" returns would have been more numerous and would have included many cases yielding strains which eventually proved to be undoubted meningococci.

As regards laboratory details, I have already referred (p. 69) to the care needed in the interpretation of negative results. The remarks of Gordon on the fermentation tests¹ remind me of further questions,

¹ *Medical Research Committee, Special Report Series. No. 3, 1917, p. 3.*

which are well worth discussing, as to the value of particular tests for determining that a given coccus is not a meningococcus. The four sugars which Gordon discusses are glucose, maltose, galactose, and saccharose. About the value of the last there is no question, as all bacteriologists are agreed that a coccus which ferments saccharose is, *ipso facto*, not a meningococcus. The other three sugars need more careful consideration.

With galactose, Gordon observes "there has been diversity of experience," and I agree with him that this is probably due "to alteration of this somewhat fragile sugar in steaming." This involves a modification of the position which Gordon held in 1907¹. He then maintained that cultural and fermentation tests were sufficient for differentiating meningococci from Gram-negative cocci of the normal throat, without resort to the agglutination test, which he was "quite unable to recommend"; and he regarded failure to ferment galactose as excluding a coccus from the meningococcus group. Now that he has very frankly changed his views in the light of subsequent research, I agree with him that it is better to abandon galactose as an exclusion test. Galactose has been given a trial in the Board's Laboratory but has not been found particularly useful. To avoid decomposition, it should be sterilised separately in 10 per cent. solution before it is added to the medium. It will then be found that the meningococcus consistently fails to ferment it. There may, however, be some advantage in using laevulose instead of galactose. This sugar, which also requires careful treatment to avoid decomposition, is not fermented by the meningococcus, but it forms acid with some strains of *flavus* which fail to attack saccharose.

With both glucose and maltose Lingelsheim found that all his strains of meningococci agreed in giving a well-marked acid reaction; and apparently Gordon's experience, as recorded in his earlier work, was the same. If the experience of other observers were in agreement on this point, there would be no disadvantage in omitting the use of maltose, as Gordon has done in his later investigations. But, as I pointed out in my previous report (pp. 408-12), several bacteriologists have found that some strains of meningococci attack maltose much more strongly than glucose; and this fact has been repeatedly confirmed in the Board's Laboratory. Not infrequently a freshly isolated strain of cerebro-spinal origin has failed to give any acid reaction in the glucose tube; a similar result with a naso-pharyngeal strain would

¹ Report to the Local Government Board on the Micrococcus of Epidemic Cerebrospinal Meningitis and its Identification.

obviously not justify the exclusion of the latter organism from the class of meningococci. So I think the use of maltose should be retained.

The above considerations indicate that the fermentation tests require careful handling, and that caution is needed in the interpretation of their results, especially when freshly isolated strains are under investigation. When the requisite precautions are taken, these tests must still be regarded as a useful part of the series of cultural tests which determine whether an organism is a meningococcus.

In 1907 Gordon was strongly of opinion that cultural tests were sufficient for the diagnosis of meningococci from the naso-pharynx, and that nothing was to be gained by supplementing them with serological reactions. I am inclined to agree with this view, provided that the cultural tests are rightly conducted. Since 1907 a large amount of agglutination work has been put on record; and when one takes a broad view of its results, instead of focussing attention upon some particular hypothesis as to serological grouping, it is seen that serological tests afford no basis for excluding from the class of meningococci an organism which has been properly identified by cultural tests as belonging to this species. This view is expressed even more strongly by the Special Advisory Committee which reported to the Medical Research Committee in 1916¹. Discussing naso-pharyngeal cocci, they say: "We should regard a meningococcus-like organism which gave *all* the cultural reactions of the meningococcus as certainly capable of producing meningitis."

In the same paragraph the Committee say:

...it appears to us that the meningococcus is shown to be a good enough "species" in the natural history sense, as species go amongst bacteria. That is to say it can be adequately separated from other Gram-negative cocci by the exercise of reasonable care. By serological means it can be divided up, it is true, into certain immunological races or strains, as will be mentioned a little later, but this need not affect its specific entity.

On p. 15, where they discuss the diagnostic value of serological reactions, they remark:

The German observers endeavoured to prove that the strains found in the throats of non-contacts were "pseudo-meningococci," but they were unable to frame any definition of a pseudo-meningococcus which would not include some undoubted spinal strains. No serum has ever been produced which will certainly distinguish between the genuine organism and the so-called pseudo-meningococcus.

¹ *Medical Research Committee. Special Report Series. No. 2, p. 10.*

And they add:

The evidence which has so far accumulated suggests that comprised under the term meningococcus there are a number of races, differing in their immunological reactions, some apparently more virulent than others, but there is so far no justification for asserting any to be destitute of potential pathogenic powers. How sharply defined and stable these races may be we do not at present know.

The above opinions, it appears to me, present a cautious and accurate review of the position established at the beginning of 1916; and their accuracy is confirmed by the results of subsequent work in the Board's Laboratory, as shown in the present series of reports.

To recapitulate, cultural tests are sufficient for the diagnosis of the meningococcus; confirmation by serological tests, which indicate a subdivision of meningococci into different serological races, is not necessary for deciding whether an organism belongs to the meningococcus species.

This position is quite compatible with the view, now held by the majority of investigators who have studied the subject, that the serological characteristics of meningococci are of considerable interest and importance; it is at variance only with the opinion of extremists who maintain that submission to a particular set of serological tests is the necessary criterion for deciding whether an organism is or is not a meningococcus.

From his reports on the recent epidemic, it is evident that Gordon has radically changed the opinions he expressed in 1907 as to the value of serological reactions. He now attaches high importance to these tests, and has used them as a basis for the subdivision of meningococci into "types." His reports for 1915 have been reviewed in the Medical Research Committee's Report, which expresses (pp. 59—60) the following conclusions as to the value of his serological work:

The "types" defined by Major Gordon by means of the agglutinin absorption test were all from the meninges and had caused epidemic cerebro-spinal fever; that is to say, they are the "epidemic races" which were mainly concerned in the outbreak in England in 1914—15. But when Gordon applied his test to pharyngeal strains, he found that only a portion of them were to be included in his types; it is possible that the residue were non-epidemic and less harmful races.

In pursuit of this last remark, the report develops a theory that meningococci may be divisible into "epidemic" and "domestic" strains. It says:

We may conceive this organism [the meningococcus] to be essentially a saprophyte, though with potentialities of parasitism, divided up, as most bacterial species probably are, into a number, perhaps a large number, of races distinguished by their

immunological reactions. At ordinary times, when cerebro-spinal fever is not epidemic, the saprophytic spread of these races is attended only by the development here and there of sporadic cases of declared disease in the most susceptible elements of the population—the posterior basic meningitis of infants. But from time to time, and hitherto very rarely in this country, individual races attain a greater virulence and their saprophytic spread is attended not only by a larger number of cases of meningitis, but by the attack of young adults, who in ordinary circumstances are immune. Such epidemic strains may be introduced into a community and lead to an outbreak of cerebro-spinal fever; there seems some ground for the belief that at least one out of the three principal strains concerned in last year's epidemic was introduced by the Canadian troops. In any given epidemic there will occur a saprophytic spread of the epidemic strains side by side with the domestic and relatively harmless strains indigenous to the locality, so that there are carriers of either, indistinguishable except by serological means. Major Gordon suggests that only those carriers need be isolated who bear epidemic strains.

This conception of epidemic cerebro-spinal fever, already we believe held by many epidemiologists, must at present be regarded as a working hypothesis only.

In short, the view of the Medical Research Committee's Report is that serological differences may be correlated with differences of virulence and may therefore be of importance in distinguishing highly dangerous from less dangerous naso-pharyngeal strains. This suggestion is interesting, and, if serologically "epidemic" strains were rare except amongst direct contacts, isolation based on the segregation of "epidemic" carriers and release of "domestic" carriers might sometimes be feasible and possibly useful. But the fact is that so-called "epidemic" strains are not rare; they are common even in the non-contact population, amongst which they seldom give rise to cerebro-spinal fever. So it is difficult to see that they are really much more dangerous than the "domestics."

This view of the Medical Research Committee appears, however, to differ very considerably from Gordon's. In his later report (1917) Gordon insists that naso-pharyngeal strains which cannot be identified serologically by means of one or other of his four monovalent sera, prepared from his four "types" of cerebro-spinal meningococci, are not to be regarded as meningococci, though they conform to the cultural and fermentation tests for the meningococcus. He terms them "non-meningococci" or "pseudo-meningococci." He thus disagrees with the opinion of the Medical Research Committee that the meningococcus can be identified by cultural tests alone, and ignores their suggestion that strains not conforming to his serological tests are merely less important varieties of that organism, because less directly associated with outbreaks of cerebro-spinal fever in its epidemic form.

Not only does he ignore this very conciliatory suggestion, evidently expressed as an appreciation of his work, but he seems definitely to repudiate it. He firmly takes his stand on the dictum that cocci not responding to his serological tests are not meningococci. By so doing, I think he places himself in serious difficulties. If, whilst rejecting the compromise suggested by the Medical Research Committee, he had taken his stand on the claim that a coccus cannot be authenticated as a meningococcus unless it agglutinates with a serum prepared from a cerebro-spinal strain, his case would have been difficult but at least it would have been arguable. But one cannot argue over a merely personal dictum, which, expressed as a syllogism, would run: all meningococci must agglutinate with a serum prepared from a cerebro-spinal strain; certain naso-pharyngeal cocci do not agglutinate with Gordon's sera prepared from cerebro-spinal strains; therefore they are not meningococci. One can only remark that the conclusion is invalid.

There is one more aspect in which it is interesting to compare the Board's results with those obtained by investigators working for the military authorities. From the beginning of 1915 onwards, the Board's pathologists have consistently found that the percentage of meningococcus carriers even amongst the general (non-contact) population is notably high. Until recently, the Army investigators have not been able to corroborate this, a circumstance which I think is readily explained by the remarks from the Medical Research Committee's Report quoted at the beginning of this appendix. The Committee states that the investigators complained of the unsatisfactory results of the methods employed for detecting the meningococcus in the naso-pharynx, and adds that "the work is tedious and beset with pitfalls, while its results were often found ambiguous." Last year, however, these reasons for dissatisfaction were evidently removed, and the investigators for the Army proceeded to find high percentages of carriers, which are quite in accordance with the previous findings of the Board. The fact that a high carrier rate had been found since the beginning of 1915 suffices to disprove the hypothesis that these later results are explicable by increase in the carrier rate.

SECOND REPORT ON THE IDENTIFICATION OF THE MENINGOCOCCUS IN THE NASO-PHARYNX, WITH SPECIAL REFERENCE TO SEROLOGICAL REACTIONS¹.

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INTRODUCTION.

IN the course of an inquiry initiated at the beginning of March, 1915, into the distribution of the meningococcus among the general population,

¹ Reprinted from *Reports to the Local Government Board on Public Health and Medical Subjects*, n.s. No. 114 (1917), by permission of His Majesty's Stationery Office.

Dr Eastwood and I had found a considerable percentage of Gram-negative cocci, indistinguishable by full cultural tests from spinal meningococci, in the naso-pharynx of out-patients attending at St Bartholomew's Hospital. These non-contact naso-pharyngeal strains were subjected by me to serological tests; and in a preliminary report¹, issued in May, 1916, it was stated that they could not be excluded from the meningococcus species on the results of the agglutination reactions. The conclusion arrived at was, in effect, that cultural and fermentation tests were sufficient for the identification of the meningococcus in the naso-pharynx. Questions of differentiation by serological methods between strains of meningococci, whether spinal or naso-pharyngeal in origin, were raised, but no final decision was made.

To summarise briefly the main features of the previous report, the evidence obtained indicated that differences in antigenic capacity afforded a basis for the division of meningococci into two fairly well-defined groups. Moreover, this serological grouping was correlated with differences in fermentative activity in the presence of maltose and glucose. As an explanatory hypothesis I suggested that differences in agglutinin production and fermentative activity are attributable to the complexity of the meningococcus antigen, which contains two components. These give rise in the animal body to two groups of agglutinins, and the differentiation into two fairly distinct serological races is due to the fact that the majority of meningococcus strains contain these components in unequal proportions, one being markedly in excess of the other. Within each group differences are to be found because the dominant type of antigen is liable to slight variations in structure; and sometimes there is no clear distinction between the one group and the other, because in a minority of strains there is no marked preponderance of the one antigen component over the other.

The work has been continued in the hope that further light may be thrown upon this differentiation of meningococci into serological races, with particular reference to the biological relationship between cerebro-spinal strains and those naso-pharyngeal strains which have been found to be of common occurrence amongst persons not associated with cases of cerebro-spinal fever. The pharyngeal strains investigated are 89 in number, including three strains of Gram-negative cocci which were distinguishable from meningococci by their pigment formation, and were used as controls. Of the 86 strains, all culturally identical with meningococci, 28 were studied in my previous report, and have been submitted

¹ Reprinted in *Journ. of Hygiene*, xv, 446.

to further investigation. The new strains, like those previously reported upon, have all been obtained from persons not known to have been associated in any way with cases of meningitis. The cerebro-spinal strains investigated are 66 in number, including 32 which were not mentioned in my last report.

In connection with the epidemic prevalence of cerebro-spinal fever, which occurred from February to May, 1915, and during approximately the same period but with a lower incidence in 1916, it may be of interest to record the periods in which the strains of meningococci were obtained. The spinal strains were derived from cases of meningitis occurring during the following periods: Cases 1-39, from March 23, 1915, to December 23, 1915; 40-66, from January 3, 1916, to September 14, 1916. The pharyngeal strains were obtained in three series from the out-patients at St Bartholomew's Hospital; 1-46, from March 29, 1915, to July 22, 1915; 47-56, from January 10, 1916, to January 24, 1916; 57-89, from April 6, 1916, to June 5, 1916.

I wish to express my indebtedness to Dr Eastwood for generous help in the preparation of this report, and to Dr Scott for much helpful criticism.

METHODS.

The usual methods of performing the macroscopic agglutination test described in my previous report have with some slight exceptions been adhered to. At first the suspensions used contained 4 mg. of moist cocci per c.c., but latterly suspensions of only 2 mg. per c.c. were preferred, as giving a sharper end-point.

All agglutinating sera were monovalent, and were prepared by inoculating rabbits intravenously with living cultures grown on glucose agar. Living culture taken from the medium immediately before inoculation appears to be less toxic than killed culture. The final readings of the agglutination tests were made after 24 hours in the incubator at 55° C., and a further 12 hours in the ice-chest or at room temperature. For absorption tests the agglutinating sera have been used in dilutions of 1:50 and 1:100. Heated and unheated cultures have been used, both in small and in large amounts. Information on these points is given in the heading of each table.

CEREBRO-SPINAL AND NASO-PHARYNGEAL MENINGOCOCCI
COMPARED IN RESPECT OF:

(1) AGGLUTINABILITY.

The agglutination tests upon spinal and pharyngeal meningococci with monovalent meningococcus sera have been summarised for the purposes of comparison in Tables I and II. So far as possible the experimental conditions in the two series have been identical.

In interpreting the results of agglutination tests upon meningococci it is necessary to bear in mind the varying agglutinability of different suspensions of the same strain prepared at different times. The causes underlying this variability are not always the same, and in some instances there is evidence of an alteration, occurring during subcultivation, in the constitution of the culture from which the suspension was prepared. In other cases the failure to form clumps appears to be a temporary phase, and it is therefore impossible to assert, when the result with a particular serum is negative, that the response to that serum will invariably be the same. With the object of eliminating disturbing factors, so far as possible, many of the agglutination tests from which my tables have been prepared have been frequently repeated; where the results have differed, the highest titre has been recorded.

As regards the method adopted, the culture suspensions, after being heated to 65° C. for $\frac{1}{2}$ to 1 hour, were diluted with 0·5 per cent. carbolic salt solution to a uniform density of 2 mg. per c.c. and were stored for several weeks before being used. The end-point chosen for comparative work is that dilution of serum in which the cocci have deposited, leaving the supernatant fluid clear or with very slight opalescence when examined in a good oblique light.

The figures in the tables represent the numerical value of the highest dilution in which the end-point was reached, the maximum recorded in the table being 1 : 1000. The titres of the six sera used were approximately equal, ranging from 1 : 800 to 1 : 1600 with different suspensions of each homologous strain. The symbols \pm and $-$ indicate respectively an incomplete and a negative result at 1 : 100; tr. indicates slight agglutination, the supernatant fluid remaining turbid.

The six monovalent agglutinating sera were prepared with strains which had been grown from the cerebro-spinal fluid of cases of meningitis occurring during the 1915 period of prevalence of cerebro-spinal fever: they were chosen because they agglutinated between them most of the spinal strains. With the exception of M 34 none of the heated sus-

TABLE I. Agglutination tests on 66 spinal (M) strains with four Group II sera and two Group I sera, prepared with spinal strains.

M Strain	Group I sera			Group II sera			M Strain	Group I sera			Group II sera		
	Serum M 10	Serum M 43	Serum M 33	Serum M 18	Serum M 24	Serum M 32		Serum M 10	Serum M 43	Serum M 33	Serum M 18	Serum M 24	Serum M 32
1	1000	1000	±	±	±	±	35	-	-	-	200	100	±
2	1000	1000	±	tr.	tr.	±	36	800	200	±	±	200	±
3	400	200	±	±	±	100	37	-	-	-	400	800	400
4	1000	1000	±	±	±	100	38	tr.	-	-	800	400	800
5	1000	800	±	±	±	100	39	-	-	-	800	400	200
6	1000	1000	±	±	±	200	40	1000	1000	1000	tr.	400	±
7	1000	400	±	±	±	100	41	1000	200	200	-	400	±
8	1000	1000	±	tr.	tr.	100	42	-	-	-	400	200	100
9	1000	1000	±	tr.	tr.	100	43	-	-	-	1000	200	±
10	1000	1000	±	tr.	tr.	100	44	-	-	-	1000	1000	200
11	800	100	±	200	100	400	45	-	-	-	1000	200	200
12	800	1000	±	100	100	±	46	-	-	-	tr.	200	±
13	800	1000	±	±	±	100	47	100	200	200	1000	200	200
14	400	100	±	±	±	100	48	1000	200	1000	1000	200	400
15	800	±	±	100	±	200	49	-	-	-	1000	200	200
16	100	100	±	±	±	200	50	200	1000	1000	-	400	400
17	400	1000	±	±	±	400	51	200	1000	1000	100	100	400
18	-	-	±	400	400	±	52	1000	1000	400	±	200	±
19	100	±	±	400	400	±	53	200	400	-	-	-	±
20	±	-	±	400	200	±	54	tr.	-	-	1000	400	400
21	400	100	±	1000	800	100	55	-	-	-	200	200	100
22	-	-	-	800	400	100	56	200	1000	1000	200	±	±
23	-	-	-	400	800	400	57	tr.	tr.	tr.	1000	100	200
24	-	-	-	1000	1000	200	58	±	±	1000	200	±	±
25	200	±	±	1000	400	100	59	200	1000	1000	400	±	±
26	-	-	-	1000	400	800	60	-	-	-	1000	400	400
27	-	±	±	1000	400	200	61	-	-	-	1000	200	400
28	-	-	-	1000	200	200	62	-	-	-	±	200	100
29	tr.	-	-	400	200	100	63	-	-	-	±	400	100
30	-	-	-	800	400	100	64	±	±	100	1000	400	1000
31	-	±	±	tr.	400	1000	65	400	100	-	±	±	100
32	-	-	-	200	400	1000	66	-	-	-	400	±	100
33	-	-	-	800	400	200							
34	1000	200	400	1000	1000	400							

The symbols ±, - and tr. refer to reactions at 1 : 100.

- = no agglutination.

± = well marked but incomplete agglutination.

tr. = trace of agglutination.

TABLE II. *Agglutination tests on 86* naso-pharyngeal (NP) strains with the same spinal sera as in Table I.*

NP Strain	Group I sera				Group II sera				Group II sera			
	NP		Serum		Serum		Serum		Serum		Serum	
	Strain	M 10	M 43	M 18	M 24	M 32	M 33	Strain	M 10	M 43	M 18	M 24
1	800	200	tr.	tr.	400	200	tr.	47	tr.	-	200	400
2	400	200	tr.	tr.	400	200	tr.	48	-	-	1000	200
3	100	400	tr.	tr.	400	200	tr.	49	tr.	100	-	400
4	400	100	-	tr.	400	400	400	50	tr.	-	1000	200
5	200	200	tr.	tr.	400	400	400	51	tr.	-	tr.	800
6	200	200	tr.	tr.	400	200	200	52	-	tr.	tr.	200
7	200	200	tr.	tr.	400	200	200	53	1000	1000	-	tr.
8	200	200	tr.	tr.	400	200	200	54	tr.	tr.	1000	200
9	200	200	tr.	tr.	400	200	200	55	tr.	tr.	tr.	200
10	200	200	tr.	tr.	400	200	200	56	tr.	tr.	tr.	200
11	200	200	tr.	tr.	400	200	200	57	tr.	tr.	tr.	200
12	200	200	tr.	tr.	400	200	200	58	tr.	tr.	tr.	200
13	200	200	tr.	tr.	400	200	200	59	tr.	tr.	tr.	200
14	200	200	tr.	tr.	400	200	200	60	tr.	tr.	tr.	200
15	200	200	tr.	tr.	400	200	200	61	tr.	tr.	tr.	200
16	200	200	tr.	tr.	400	200	200	62	tr.	tr.	tr.	200
17	200	200	tr.	tr.	400	200	200	63	tr.	tr.	tr.	200
18	200	200	tr.	tr.	400	200	200	64	tr.	tr.	tr.	200
19	200	200	tr.	tr.	400	200	200	65	tr.	tr.	tr.	200
20	200	200	tr.	tr.	400	200	200	66	tr.	tr.	tr.	200
21	200	200	tr.	tr.	400	200	200	67	tr.	tr.	tr.	200
22	200	200	tr.	tr.	400	200	200	68	tr.	tr.	tr.	200
23	200	200	tr.	tr.	400	200	200	69	tr.	tr.	tr.	200
24	200	200	tr.	tr.	400	200	200	70	tr.	tr.	tr.	200
25	200	200	tr.	tr.	400	200	200	71	tr.	tr.	tr.	200
26	200	200	tr.	tr.	400	200	200	72	tr.	tr.	tr.	200
27	200	200	tr.	tr.	400	200	200	73	tr.	tr.	tr.	200
28	200	200	tr.	tr.	400	200	200	74	tr.	tr.	tr.	200
29	200	200	tr.	tr.	400	200	200	75	tr.	tr.	tr.	200
30	200	200	tr.	tr.	400	200	200	76	tr.	tr.	tr.	200
31	200	200	tr.	tr.	400	200	200	77	tr.	tr.	tr.	200
32	200	200	tr.	tr.	400	200	200	78	tr.	tr.	tr.	200
33	200	200	tr.	tr.	400	200	200	79	tr.	tr.	tr.	200
34	200	200	tr.	tr.	400	200	200	80	tr.	tr.	tr.	200
35	200	200	tr.	tr.	400	200	200	81	tr.	tr.	tr.	200
36	200	200	tr.	tr.	400	200	200	82	tr.	tr.	tr.	200
37	200	200	tr.	tr.	400	200	200	83	tr.	tr.	tr.	200
38	200	200	tr.	tr.	400	200	200	84	tr.	tr.	tr.	200
39	200	200	tr.	tr.	400	200	200	85	tr.	tr.	tr.	200
40	200	200	tr.	tr.	400	200	200	86	tr.	tr.	tr.	200
41	200	200	tr.	tr.	400	200	200	87	tr.	tr.	tr.	200
42	200	200	tr.	tr.	400	200	200	88	tr.	tr.	tr.	200
43	200	200	tr.	tr.	400	200	200	89	tr.	tr.	tr.	200
44	200	200	tr.	tr.	400	200	200					
45	200	200	tr.	tr.	400	200	200					
46	200	200	tr.	tr.	400	200	200					

* Three pigmented strains 29, 30 and 32 used as controls are omitted from this table; they gave no agglutination.

The symbols have the same meaning as in the preceding table.

pensions of the spinal or pharyngeal strains was agglutinated in 1 : 50 by normal rabbit serum.

Table I summarises the results with the spinal strains; the first 34 strains are arranged as in the previous report to show the grouping; the remaining strains are in the serial order in which they were obtained.

On examination of Table I it will be seen that if the sera M 18 and M 24, which I call Group II, and M 10 and M 43, which I call Group I, are taken as standards, the spinal strains can be divided into two moderately well defined groups, excluding certain strains which may be classed as intermediate or indeterminate. Amongst the strains which appear to be definitely grouped are two, M 32 and M 33, which are agglutinated sufficiently well by M 18 and M 24 sera to be considered as belonging to the same group as the strains M 18 and M 24, and to a different group from M 10 and M 43. But the sera produced with M 32 and M 33 invalidate the grouping, since they on the one hand agglutinate strains not affected by M 18 and M 24 sera, and on the other hand leave unaffected strains which these same two sera agglutinated.

It is clear, therefore, that the strains M 18, M 24, M 32 and M 33, which appear to belong to the same group, though closely inter-related as regards agglutinin production and agglutinability, are not identical with each other in either respect.

A further obstacle to the division of meningococci into two or more well-defined groups is the fact that there are strains either relatively inagglutinable towards the group sera or agglutinated equally by sera of both groups.

To sum up the position as regards agglutinability, the spinal meningococci can be separated into two main groups by means of selected sera, but such division cannot be strictly maintained if sera made from unselected strains are used, since strains grouped in a particular fashion by one set of sera do not necessarily behave uniformly in relation to a different set of sera. This division into two groups must therefore be admitted to be imperfect and somewhat artificial, since it depends on the arbitrary selection of particular sera and the exclusion of other sera. Nevertheless, when these limitations are recognised, a rough division into two main groups is of great importance to a correct appreciation of the inter-relationship between strains of meningococci.

The question of grouping strains may be looked at from another point of view which indicates the principle employed in selection. Every serum which exercises a definite selective action upon strains belonging to one or other of the two groups may be considered to

contain agglutinins with special affinities for that particular group. Reviewing the method of classification used in my previous report, one finds that M 1 to M 17 were classed as Group I strains and M 18 to M 34 as Group II strains, on the ground of simple agglutinability with particular sera. From the above considerations, this may be more correctly expressed by saying that this grouping is based on a selective response to particular agglutinins, viz. a response to the predominant agglutinins produced by the antigens of M 10 and M 43 in the case of Group I and a response to the agglutinins produced by the antigens of M 18, M 24, M 32, M 33 in the case of Group II. In other words, agglutinogenic capacity would be a more scientifically accurate basis for classification, as it would provide identification of the antigens producing the special agglutinins. Agglutinability is too variable a property to be strictly reliable. It is, however, a useful guide to identification inasmuch as it effects a rough demarcation of strains into two groups. I have therefore continued to refer to particular strains as belonging to Group I or Group II when there is sufficient evidence of the predominance of one or other of the two group antigens.

Turning now to Table II, the next step in the analysis of these tables is to compare the agglutinability of spinal and pharyngeal strains in relation to the agglutinins of the above-mentioned six sera. This table, read in conjunction with Table I, shows that although an exact serological classification of meningococci into groups cannot, as shown in the previous discussion, be made upon the results of simple agglutination tests alone, important evidence of relationship is obtained which can be correctly interpreted by subsequent investigation of agglutinogenic and absorptive capacities. It will facilitate comparison of the two series of strains in respect of agglutinability if the tables are summarised in the following way. I have taken a titre which is sufficiently high to be regarded as of definite serological significance, and have ascertained the total number of strains in the two series attaining or surpassing this titre in respect of each serum. Since each of the six sera has a titre ranging from 1 : 800 to 1 : 1600 with different suspensions of the homologous strain, a complete agglutination result in a dilution of 1 : 400 may be taken to constitute important evidence of relationship. The results are summarised in the following table, which gives under the heading of each serum the percentage of spinal and pharyngeal strains agglutinated completely in a dilution of 1 : 400 or over.

TABLE III.

Summary of Tables I and II, giving the percentages of spinal and pharyngeal strains agglutinated to 1 : 400 by each serum.

Total strains	M 18 Serum	M 24 Serum	M 32 Serum	M 33 Serum	M 10 Serum	M 43 Serum
66 spinal . .	47 %	35 %	17 %	33 %	41 %	30 %
86 pharyngeal .	37 %	34 %	24 %	25 %	6 %	5 %

Comparing one series with the other as regards the total strains agglutinated by one or more of the six sera, it is found that 94 per cent. of the 66 spinal strains were agglutinated to 1 : 400 or over, and 72 per cent. of the 86 pharyngeal strains reacted to a similar extent.

It will be seen from Table III that the relative proportions of spinal and pharyngeal strains agglutinated up to 1 : 400 by the Group II sera do not differ materially. The agglutinins of M 18 and M 33 sera find among the spinal strains more combining affinities than among the pharyngeal, but the reverse is the case with M 32 serum, and M 24 serum influences spinal and pharyngeal strains in equal numbers.

In striking contrast to the above, the Group I agglutinins contained in M 10 and M 43 sera find related antigens 6-7 times more frequently among the spinal than among the pharyngeal strains.

Two alternative explanations may be suggested to account for this difference in reaction between naso-pharyngeal and cerebro-spinal strains in the presence of a Group I serum:

(1) Group I antigen is a rare component of the pharyngeal meningococcus of the non-contact, while it is represented as commonly as Group II antigen in strains of spinal meningococci. Thus, if the proportions found in this investigation reflect the general distribution of the two groups, both in meninges and naso-pharynx, it may be inferred that the meningococcus of Group I more frequently invades the meninges from the naso-pharynx than the meningococcus of Group II.

(2) The antigen of Group I in the pharyngeal meningococcus, though not revealed by the two Group I sera used, might be shown to have combining affinities for agglutinins produced by other spinal strains containing Group I antigen.

Some evidence in regard to this second hypothesis is furnished by agglutination tests with sera of Group I prepared with M 15, M 16, and M 17.

M 17 serum, titre 1 : 800 to 1 : 1600, was found to agglutinate completely NP 4 and NP 5 up to 1 : 800; NP 3, 17, 53, 65, 70, 71, up to 1 : 400; NP 67 and 86 up to 1 : 200.

M 15 serum, titre 1 : 400, agglutinated completely in 1 : 100, NP 19, 21, 24, 44, 52, 56, 60, and 70.

M 16 serum, titre 1 : 800 to 1 : 1600, agglutinated NP 2 to 1 : 800, NP 70 and 71 to 1 : 400, and NP 74 to 1 : 200.

A further point brought out by comparison of Tables I and II is that the number of spinal strains not agglutinated by any of the six spinal sera up to 1 : 400 was 4 (6 per cent.), while the corresponding number of pharyngeal strains was 24 (28 per cent.). This clearly constitutes a distinction of some importance between the spinal and the pharyngeal strains. But failure to attain an arbitrary standard of agglutination on the part of certain pharyngeal strains is no proof that they are specifically different from meningococci of proved pathogenicity, since on the same grounds a proportion of the latter would also be excluded from the meningococcus species.

It is possible that the spinal strains which were agglutinated weakly by the selected spinal sera have other serological features in common with the similarly reacting pharyngeal strains. The relationship of these strains to each other and to other spinal strains will be considered later in the light of the more precise knowledge of the combining qualities of antigens which can be gained from the production of agglutinating sera and from agglutinin absorption experiments.

The general result of the comparison of naso-pharyngeal strains with cerebro-spinal in respect of agglutinability may be summed up as follows:

(1) The agglutinins of Group II in the sera produced by M 18, M 24, M 32, and M 33 combine in almost equal proportions with spinal and pharyngeal strains.

In contrast, the agglutinins of Group I in the sera produced by M 10 and M 43 have more combining affinities with spinal strains than with pharyngeal.

(2) Strains which have little capacity for combining with the agglutinins of either Group I or Group II in the six spinal sera are found between four and five times more often in the pharyngeal series of meningococci than in the spinal.

(2) AGGLUTINOGENIC CAPACITY.

In the preceding section, which deals with simple agglutination tests, under identical conditions, on cerebro-spinal and naso-pharyngeal strains, six monovalent agglutinating sera prepared with spinal strains were employed. Two of these sera, M 10 and M 43, were found to exer-

cise a selective action upon one group of strains, while the two sera, M 18 and M 24, influenced a second group which were not agglutinated by the first two sera. The strains M 10 and M 43 evidently possess antigenic capacities which differ from those of M 18 and M 24. The agglutinins which each of these four strains produces are capable of combining with similar antigens in other strains, but the reaction of agglutination resulting from such combination is not necessarily evidence that the antigens concerned are equally represented in the strain agglutinated and in the strain producing the agglutinin. For example, the strains M 32 and M 33 are agglutinated by the Group II sera M 18 and M 24, but the sera which they produce are by no means identical either with the latter or with each other in their selective action upon the whole series of spinal meningococci (see Tables I and II). In my previous report a more marked illustration was given of the absence of correspondence between agglutinability and agglutinogenic capacity. Two strains, NP 10 and NP 11, were both agglutinated by the same Group II sera, and were almost identical in agglutinability. While the serum produced with NP 10 agglutinated exclusively the spinal strains of Group II, NP 11 serum agglutinated mainly the Group I spinal strains.

The above evidence indicates that agglutinogenic capacity sometimes brings to light affinities not demonstrable by simple agglutination between the antigens possessed by different strains. Further observations have been made with a view to ascertaining whether the complete antigenic capacities of the various strains are revealed by the tests of agglutinability with the six selected sera, the results of which are summarised in the first two tables. Certain pharyngeal and spinal strains were selected for the preparation of agglutinating sera. The results of tests with these sera on spinal and pharyngeal strains are set out in Tables IV, V, and VI. There is a difference in technique which must be noted in comparing these with the preceding tables. In the preceding tables the strength of the suspensions was 2 mg. per c.c., but in these it was 4 mg. per c.c. throughout, and this alteration approximately doubles the apparent agglutinating titre.

Analysis of Table IV.

The sixteen naso-pharyngeal strains, NP 31-NP 46, represent the positive findings (except NP 32) from about 200 consecutive throat swabs. They form an unselected series which may be taken as an average sample of non-contact meningococci. NP 32, included as a

TABLE IV.

Agglutination tests on an unselected series of 16 naso-pharyngeal strains with a Group I spinal serum and a Group II spinal serum and with sera made from 8 naso-pharyngeal strains.

Strain	M 23 Serum	M 9 Serum	NP 10 Serum	NP 11 Serum	NP 36 Serum	NP 39 Serum	NP 40 Serum	NP 41 Serum	NP 43 Serum	NP 44 Serum
NP 31	-	-	-	200	400	±	±	±	±	400
NP 32*	-	-	-	-	-	-	-	-	-	-
NP 33	100	±	±	100	200	-	-	100	-	±
NP 34	400	-	100	200	-	±	±	-	±	400
NP 35	200	-	200	±	-	±	-	-	-	±
NP 37	200	-	200	-	-	-	-	-	-	-
NP 38	400	-	200	100	-	±	-	-	±	100
NP 36†	200	±	100	1000	800	100	100	100	200	200
NP 39	-	±	-	100	100	400	200	±	100	800
NP 40	-	200	-	200	±	200	400	±	200	400
NP 41	-	±	±	1000	200	-	200	800	200	±
NP 42	-	-	-	800	200	±	-	-	100	200
NP 43	-	100	±	1000	200	200	200	400	400	800
NP 44	-	-	±	200	-	100	100	±	200	400
NP 45	1000	-	400	200	100	200	±	±	±	200
NP 46	-	-	-	-	-	-	-	-	-	-

* Pigmented Gram-negative coccus.

† NP 36 has been removed from its serial order to make clearer the tendency to grouping.

control, was a golden yellow pigmented culture of a gram-negative coccus with the fermentation reactions of the meningococcus.

Orientation tests were made with two spinal sera upon the pharyngeal suspensions, which were the same throughout, and the results are given in the second and third columns. M 9 serum contains agglutinins of Group I, and M 23 serum agglutinins of Group II. It will be seen from the table that seven of the pharyngeal strains are agglutinated by the Group II serum and two by the Group I, the remainder not responding to the action of either serum.

In the light of these results, certain of the pharyngeal strains were selected for the production of sera, preference being given to those which were not agglutinated to any marked degree by either of these two spinal sera¹. The two sera made with NP 10 and NP 11, referred to above, were included for comparison.

The results of these tests, as shown in the table, are that, with the exception of NP 10 serum, none of the pharyngeal sera agglutinates

¹ Later tests, however, showed that several were agglutinated by other spinal sera of Group II, M 18 and M 24. (See Table II.)

the same collection of pharyngeal strains as the two standard spinal sera. NP 10 has almost the same selective action as M 23 serum (Group II). The agglutination results with the other pharyngeal sera appear at first sight irregular, but present the following features of significance:

(1) They generally fail to agglutinate those pharyngeal strains which were agglutinated by the standard Group II serum (M 23).

(2) They are fairly consistent in agglutinating the strains not affected by M 23 serum.

(3) They agglutinate NP 40 and NP 43, the only two strains in this series affected by the standard Group I serum (M 9).

(4) They therefore differ from Group II, as represented by M 23 antigen, and appear to resemble Group I, as represented by M 9 antigen.

In order to throw further light on the characters of these pharyngeal strains I have tested the sera prepared with them upon two series of spinal strains representative of Group I (Table V) and Group II (Table VI).

TABLE V.

Agglutination tests on spinal strains of Group I with sera prepared from six naso-pharyngeal strains, and from two spinal strains of relatively feeble absorptive capacity.

Strain	M 16 Serum	M 46 Serum	NP 39 Serum	NP 40 Serum	NP 41 Serum	NP 43 Serum	NP 44 Serum	NP 36 Serum
M 1 . .	±	800	400	200	200	200	400	200
M 2 . .	200	200	200	100	200	±	200	200
M 3 . .	100	200	200	200	200	100	400	200
M 4 . .	±	200	400	200	200	200	400	200
M 5 . .	±	200	400	200	200	200	200	200
M 7 . .	±	200	200	200	200	100	100	200
M 8 . .	100	200	400	200	200	200	400	±
M 9 . .	200	200	200	200	200	200	400	200
M 10 . .	±	200	100	200	200	100	200	200
M 11 . .	200	200	200	400	400	100	400	200
M 12 . .	±	—	200	400	100	200	200	200
M 13 . .	100	—	200	100	±	±	400	200
M 14 . .	±	100	100	100	400	100	200	200
M 15 . .	±	—	±	200	800	100	200	200
M 16 . .	*±	100	±	±	400	100	200	100
M 17 . .	±	±	200	200	100	100	400	400
M 36 . .	400	±	100	200	400	100	400	100
M 40 . .	±	±	200	400	100	200	400	400
M 41 . .	200	100	200	200	400	200	400	200
M 43 . .	200	100	100	100	200	±	400	200
M 46 . .	±	100				200	±	±

* Agglutination marked but incomplete up to 1 : 400.

TABLE VI.

*Agglutination tests on spinal strains of Group II to
compare with Table V.*

Strain	M 16 Serum	M 46 Serum	NP 39 Serum	NP 40 Serum	NP 41 Serum	NP 43 Serum	NP 44 Serum	NP 36 Serum
M 18 . .	±	±	200	±	±	±	200	-
M 19 . .	±	-	±	-	±	±	±	-
M 20 . .	-	-	±	-	-	±	±	-
M 21 . .	800	400	200	-	400	±	200	100
M 22 . .	-	±	200	100	100	200	200	100
M 23 . .	-	±	±	100	±	-	100	±
M 24 . .	±	±	±	±,	-	-	±	-
M 25 . .	±	100	±	-	±	100	±	±
M 26 . .	-	±	100	100	±	±	100	100
M 27 . .	±	-	±	-	±	±	100	±
M 28 . .	±	-	-	-	-	-	±	-
M 29 . .	±	±	±	-	±	±	200	-
M 30 . .	-	-	±	100	-	-	±	-
M 31 . .	±	-	±	-	±	-	±	-
M 32 . .	±	-	-	±	-	-	±	-
M 33 . .	100	±	±	±	-	-	200	±
M 34 . .	±	±	-	-	200	-	100	±
M 35 . .	-	±	±	-	±	-	±	-
M 37 . .	±	±	±	±	±	±	200	-
M 38 . .	-	±	±	100	±	-	100	-
M 39 . .	-	-	100	-	-	±	100	±
M 42 . .	±	-	±	-	-	-	±	±
M 44 . .	±	±	±	±	-	100	±	-
M 45 . .	100	100	100	±	±	100	200	±

These tables (V and VI) show the following results with the sera prepared from the six strains NP 36-44:

(a) Good agglutination with cerebro-spinal meningococci, though usually short of full titre.

(b) More uniform influence on Group I strains.

(c) Agglutination of some of the Group II strains to half full titre.

It will be observed that: (a) shows the relationship of these strains to cerebro-spinal meningococci; (b) confirms their antigenic relationship to Group I, already suggested by M 9 serum above; (c) shows their relationship to Group II antigens, which was not brought out by M 23 serum, but had been indicated (Table II, p. 129) by agglutination tests with other Group II sera (M 18, etc.).

Tests of agglutinogenic capacity therefore show that some of these naso-pharyngeal strains, *e.g.* NP 44, possess both Group I and Group II antigens. As a parallel to this interesting fact, I call attention to the

following details of tests with three spinal sera, two of which are included in Tables V and VI. In agglutinogenic capacity the strains M 16, M 46 and M 55, used to produce the sera, appear to have features in common with the above-mentioned pharyngeal strains.

M 16 was agglutinated to 1 : 100 with M 8 serum (Group I), and at first absorbed the homologous agglutinin well, but not completely. After prolonged subculture it retained its slight agglutinability towards Group I sera, and was also agglutinated by certain Group II sera, but exhibited no absorptive capacity for sera of either group. After repeated inoculations of living culture into a rabbit, a serum was obtained which agglutinated the homologous strain incompletely in from 1 : 100 to 1 : 400. Later the homologous strain became much more agglutinable, giving a complete reaction to the same serum in 1 : 800 to 1 : 1600. The results of the agglutination tests show it to be an indifferent serum of low multivalency, related slightly to both groups. M 46 was obtained from basal meningitis in an infant, and in its relative inagglutinability resembled certain pharyngeal strains previously mentioned. Although M 46 was agglutinated a little by M 24 serum (Group II) and not by the two Group I sera, its agglutinogenic capacity reveals a closer relationship to the spinal strains agglutinated by Group I sera.

M 55 was another cerebro-spinal strain which, like the above two, could not be identified with either of the two groups which form the majority; it was obtained from a case of post-basal meningitis in a child aged eight months. It was agglutinated slightly by Group II sera. A serum was prepared from it and tests were made upon the spinal strains. The agglutination results, which are not given in the table, are as follows. The homologous strain was agglutinated completely in a dilution of 1 : 800, and M 31 to 1 : 400; none of the other spinal strains tested, whether of Group I or Group II, was agglutinated higher than 1 : 100. Brief reference may be made to the reactions of two of the remaining pharyngeal strains.

NP 31 had poor antigenic qualities and produced a serum with no higher titre than 1 : 200 for the homologous culture. This serum agglutinated only two of the spinal strains of Group II up to 1 : 200 and none of the Group I strains.

NP 46 culture was tested with a large number of spinal and pharyngeal sera, and seemed to have no combining affinities until it was tried with M 18 serum (titre about 1 : 1600) which agglutinated it completely up to 1 : 800. Its agglutinogenic capacity was not tested.

Summary of Agglutinogenic tests.

The following inferences are to be drawn from the above results.

(1) Agglutinability does not invariably correspond with agglutinogenic capacity.

(2) The production of an agglutinating serum, *i.e.* the demonstration of agglutinogenic capacity, confirms the evidence of specific relationship between spinal and pharyngeal strains based on the agglutination tests with standard meningococcus sera.

(3) In both the cerebro-spinal and the naso-pharyngeal series, strains occur in which there is no marked predominance of one group antigen over the other.

(4) Compared with the standard spinal strains these pharyngeal strains with the characters described above may be summed up as being less well defined in relation to the two main groups both in agglutinability and in agglutinogenic capacity. In this respect there is a close analogy between them and the anomalous spinal strains, M 16, M 46 and M 55.

(3) AGGLUTININ ABSORPTION.

The preceding experiments on agglutinability and agglutinogenic capacity have shown that cerebro-spinal and naso-pharyngeal meningococci possess antigenic substances common to both classes, and that the serological differences which can be demonstrated between spinal and pharyngeal strains are not greater either qualitatively or quantitatively than the differences between individual spinal strains.

It is recognised that an antigen may be the common constituent of related organisms which have specifically distinct pathogenic properties, and that agglutinability in itself is not conclusive evidence of identity. On the same grounds, but to a less degree, the evidence supplied by agglutinogenic capacity may not always be conclusive. A strain of gonococci, for example, may produce a serum which agglutinates some strains of meningococci. The final criterion of serological identity is the absorption of agglutinin. This test provides more precise information as to the combination of agglutinin and antigen than the simple agglutination test alone, and it defines more clearly than the agglutinogenic test the degree of relationship between the antigens of different strains.

The absorption tests have been carried out on the following plan. In the first place the relationship between the different spinal strains has been worked out, and several varieties of antigens and agglutinins

have been defined in each of the two main groups. The results have then been applied to the identification of such combining affinities in the naso-pharyngeal strains and sera as are similar to the affinities exhibited by the antigens and agglutinins of the spinal strains.

A uniform method of absorption has not been adopted: the quantities and condition, *i.e.* heated or unheated, of the culture employed for absorption, and the dilutions of the absorbed serum have been varied and will be noted under each series of experiments. The experiments have been arranged in two series following the rough classification into two groups indicated by agglutination, on the assumption that there are two primary antigenic substances in the meningococcus and that the agglutinins produced by one substance or its variations cannot combine with the other.

Agglutinin absorption with spinal strains of Group I.

The method adopted was to bring together equal parts of a 1 in 25 dilution of serum and a heated suspension containing 4 mg. of cocci per cubic centimetre. After absorption, which was generally allowed to take place at room temperature, the serum was titrated and tested in dilutions of 1 in 100 up to the full titre on the strain with which the serum had been prepared (referred to as homologous). Specific absorption of agglutinin was considered to have taken place when the agglutinating power of the absorbed serum was definitely reduced for the homologous strain. The standard of reduction was that produced by the homologous strain itself under the same conditions.

The sera used in this series were those which contained mainly the agglutinins of Group I. For absorption, 21 strains all agglutinated by these sera were selected from the first 46 spinal strains. To these M 46 was added, because, though not agglutinated by these sera, it was shown to produce agglutinins for Group I strains. The number of strains used was limited in the first instance to these 22, in order that the test with each serum might be completed on a single occasion, and that the conditions of each experiment should be identical for all the strains concerned.

The absorption tables are arranged as follows. In the first column are the strains used for absorbing the serum. The succeeding columns show the results of testing, in the dilutions given at the head of each column, the agglutinating action of the serum upon a test suspension, generally that of the strain producing the serum. The first test is with the unabsorbed serum; the rest show how this initial titre is affected

by the treatment with each of the strains used for absorbing. In Table VII an absorption experiment with M 10 serum is given in full. It consists of two parts, showing the effect of two successive additions of culture. As will be seen, certain of the 22 strains removed varying amounts of the agglutinin which acts upon M 10, while others left this agglutinin unaffected.

The result with M 6 shown in Table VII is exceptional. At the time when the culture for absorption was made this strain had lost its agglutinability and absorbing capacity, which were originally the same as the other strains in the first ten. On returning to an older subculture and retesting, the original absorbing capacity was exhibited.

TABLE VII.

M 10 serum absorbed with 22 spinal strains. Absorbed and unabsorbed serum titrated and tested upon the same suspension of the homologous strain.

Method. First absorption: equal parts of 1 : 25 dilution and suspensions containing 4 mg. of heated culture per c.c. Second absorption: each suspension again added to the absorbed serum now in 1 : 50 dilution.

Absorbing Strain	Test on M 10 After 1st absorption				Test on M 10 After 2nd absorption			
	200	400	800	1600	200	400	800	1600
*Nil	.	.	+	+	+	+	+	±
M 1	.	.	+	+	+	+	-	-
M 2	.	.	+	±	±	-	-	-
M 3	.	.	+	+	-	-	-	-
M 4	.	.	±	-	-	-	-	-
M 5	.	.	±	-	-	-	-	-
M 6	.	.	+	+	+	+	+	±
M 7	.	.	+	-	tr.	-	-	-
M 8	.	.	+	±	-	-	-	-
M 9	.	.	+	+	tr.	-	-	-
M 10	.	.	+	+	±	-	-	-
M 11	.	.	+	+	+	+	+	±
M 12	.	.	+	+	+	+	+	+
M 13	.	.	+	+	+	+	+	±
M 14	.	.	+	+	+	+	+	±
M 15	.	.	+	+	+	+	+	±
M 16	.	.	+	+	±	+	+	±
M 17	.	.	+	+	+	+	+	±
M 36	.	.	+	+	+	+	+	±
M 40	.	.	+	+	tr.	-	-	-
M 41	.	.	+	+	+	+	+	±
M 43	.	.	+	tr.	-	-	-	-
M 46	.	.	+	+	+	+	+	+

* Nil signifies that the test on M 10 suspension is with the control portion of M 10 serum treated like the others except that no culture was added.

In this and succeeding tables + = complete agglutination, ± = incomplete agglutination, - = no agglutination, tr. = trace of agglutination.

Sera were prepared with some of the strains which absorbed and with others which failed to absorb, and have been used to test in a similar manner the absorbing capacity of the whole series of 22. The extent to which the agglutinin was removed varied with different sera, but in general the ease with which the agglutinating power of a serum with a titre of over 1:1000 was almost completely annulled by small amounts of culture was characteristic of sera of this group.

For the sake of brevity I have not reproduced in detail the tables recording the tests with the other sera, but have summarised the results. In the following scheme I have taken the 22 strains, and shown that they differ in range of absorptive capacity (indicated by a thick horizontal line) when tested against six sera.

Strains used for absorption		Sera absorbed				
		M 1, 8, 9, 10 containing A agglutinin	M 15 containing C agglutinin	M 17 containing B agglutinin	M 16	M 46
	M 10, 40, 43	—————	—————	—————		
(1) ...	M 1—9	—————	—————	—————		
(2) ...	M 12, 13, 17		—————	—————		
(3)	M 11, 14, 15, 36, 41		—————			
	M 16				—————	
	M 46					—————
(1) Sub-group A.		(2) Sub-group B.		(3) Sub-group C.		

Strains 11, 14, 15, 36, and 41 remove only the agglutinin from M 15 serum, which must therefore contain an agglutinin with a special combining affinity for a particular antigen in that group of strains. This agglutinin may be designated C.

Strains 12, 13, and 17 remove not only M 15 agglutinin, but, unlike 11–41, also M 17 agglutinin. This group must therefore contain an additional antigen with a combining affinity for a different agglutinin in M 17 serum. The latter may be designated B.

Strains 1–9 agree with 11–41 in removing C agglutinin and in failure to remove B agglutinin, but differ from both 11–41 and 12–17 in removing the agglutinin from the sera M 1, 8, 9, and 10, which were not affected by either of the two last mentioned groups of strains. These sera, M 1–10, must therefore contain an agglutinin, not present in sera M 15 or M 17, which may be designated A.

Strains 10, 40, and 43 remove both A, B, and C.

Strains 16 and 46 remove neither A, B, nor C, but only the agglutinin from their own sera. They are put in Group I because they agglutinate Group I strains better than Group II (see pp. 136–7).

The special characters of the various Group I sera used in the above summary require some annotation.

M 1 serum had a titre of 1 : 1600 and was prepared after the strain had been sub-cultivated for nearly a year. A definite distinction between the strains respectively absorbing and non-absorbing was brought out by agglutination tests alone with this serum, which was not the case with the other sera of this sub-group A. Absorption was well marked, the titre being reduced from 1 : 1600 to less than 1 : 100, and the same strains which absorbed the homologous agglutinin also absorbed from M 1 serum the agglutinin which acted on M 10 and M 43.

M 8 serum had a titre of 1 : 800. While there was not brought out by simple agglutination the same differentiation as above between the absorbing and non-absorbing strains, the differences in absorptive capacity were well marked, not the least reduction being effected by the latter, while a large reduction was produced by the former.

M 9 serum, with a titre of 1 : 800, was not so readily absorbed as the two preceding sera. M 1 only reduced the agglutinating power from 1 : 800 to 1 in 400. Not the least reduction was effected by the non-absorbing strains.

M 17 serum, titre 1 : 800, showed a trace of reduction in the highest dilutions with the negative strains and complete absorption with the positive, excepting M 43. This strain on some occasions absorbed well and on others very little.

M 15 serum had a titre of 1 in 400 and this only as the result of prolonged immunisation of the rabbit. Absorption was almost complete with all 20 strains, excepting M 1, with which it was slight. The non-absorbing strains, M 16-M 46 and the inagglutinable strain of M 6, made not the least impression.

Absorptive capacity in relation to the quantity and physical condition of the culture used for absorption.

The question must be considered whether, since the above grouping was effected by the use of minimal quantities of absorbing culture, the differences in absorptive capacity are to any extent merely quantitative.

Further experiments have been made with the above-mentioned 22 strains upon Group I sera, to ascertain the effect of absorbing with larger amounts of culture and with unheated culture. The results are as follows, under the head of each serum:

M 1 serum. Suspensions increased to 30 mg. per c.c. and added to equal parts of 1 : 25 serum showed practically no increased absorption

with the non-absorbing strains. Living culture added directly to the serum, the growth from a whole glucose agar tube to 1.5 c.c. of 1 in 50 dilution, gave little increase with any but M 12, which absorbed completely.

M 9 serum. The suspensions were increased to 20 mg. per c.c.; the absorption results were as before.

In an absorption experiment with M 10 serum the living growth from a whole slope (about 30–40 mg.) of each of the strains, M 14, 15, 36, and 41, was added to 1.5 c.c. of 1 : 50 dilution without effecting the least reduction of the agglutinin acting on M 10, although these strains were agglutinated to a high titre by this serum.

M 17 serum. The addition of a whole living slope culture to 1 c.c. of 1 : 25 dilution caused complete absorption in the case of M 10, 12, 13, 17, 40, and 43, as before; there was very slightly increased absorption with some of the other strains, but none with M 11, 36, and 41. A whole slope culture in 1 c.c. of 1 : 50 dilution gave a similar result. Still larger quantities up to two tubes per c.c. of 1 : 50 caused some reduction with M 1–9, but none with M 11, 36, 41, and 46.

The above experiments confirm the previous conclusion that the agglutinins in the various Group I sera with which the tests were made belong to three varieties with different combining qualities.

*Additional observations on absorptive capacities of
Group I spinal strains.*

As shown in the tabular summary, p. 142, three of the 20 strains which absorbed from one or other of the Group I sera were found to combine with all three agglutinins, designated A, B, and C. Sera were available from two of these strains, M 10 and M 43, and it is interesting to find that at least two of the agglutinins can be demonstrated in each of the sera, *i.e.* there is a correspondence between absorptive and agglutinogenic capacity. Some experiments with M 10 and M 43 sera will now be described. They show incidentally that changes in the absorptive capacity of certain strains occurred during cultivation; in addition, these experiments serve to complete the classification of the remaining spinal meningococci and to define more accurately their position in the series in which Group I antigen predominates.

M 43 serum.

In Table VIII two separate experiments with M 43 serum are recorded.

TABLE VIII.

M 43 serum absorbed with 22 spinal strains. Titre of absorbed and unabsorbed serum tested on M 43 (different suspensions, A and B).

Method. Experiment 1: 2 mg. of culture (heated suspension) per c.c. of 1 in 50. Experiment 2: growth from a whole tube, 30-40 mg., added to 1.5 c.c. of 1 in 100 serum.

Absorbing Strain	EXPERIMENT 1.				EXPERIMENT 2.			
	Test on M 43. Suspension A				Test on M 43. Suspension B			
	200	400	800	1600	200	400	800	1600
*Nil . .	+	+	+	+	+	+	+	±
M 1 . .	±	±	-	-	±	±	±	tr.
M 2 . .	+	+	tr.	-	±	±	±	tr.
M 3 . .	+	+	+	tr.	±	±	±	±
M 4 . .	±	-	-	-	±	±	±	-
M 5 . .	±	±	-	-	±	±	±	-
M 6 . .	+	+	+	+	+	+	±	±
M 7 . .	+	±	tr.	-	±	±	±	tr.
M 8 . .	±	±	-	-
M 9 . .	+	±	±	-	±	±	tr.	-
M 10 . .	+	±	tr.	-	+	+	±	±
M 11 . .	+	+	+	±	±	±	±	tr.
M 12 . .	+	+	+	+	-	-	-	-
M 13 . .	+	+	+	+	-	-	-	-
M 14 . .	+	+	+	+	-	-	-	-
M 15 . .	+	+	+	±	±	±	±	tr.
M 16 . .	+	+	+	±	±	±	±	tr.
M 17 . .	+	+	+	+	-	-	-	-
M 36 . .	+	+	+	±	±	±	±	±
M 40 . .	+	+	±	-	-	-	-	-
M 41 . .	+	+	+	+	±	±	±	tr.
M 43 . .	+	±	tr.	-	-	-	-	-
M 46 . .	+	+	+	+

* In this and succeeding tables Nil signifies that the test is upon unabsorbed serum.

The first experiment shows that the homologous agglutinin has been reduced by certain strains. A further investigation (not recorded in the table) was then made in order to render the experiment more complete, as in the case of M 10 serum (Table VII). Each heated suspension was again added to the absorbed dilutions, now 1 : 50. The test of this second absorption (not reproduced) was made on a different suspension of M 43 from the one used for the first. The result was unexpected. Instead of the agglutinating power of the serum being further reduced by those strains which absorbed after a single application of culture, it was in some cases apparently increased. On the other

hand, certain strains, *e.g.* M 12, 13, and 17, which had not at first exhibited any combining affinity for the homologous agglutinin, after the second addition absorbed the whole.

In the second experiment of Table VIII the absorption of M 43 serum was repeated with larger amounts of living culture added to the serum diluted to 1 : 100. Again the result was different from that in the first half of the table. Two strains, M 40 and M 43, which had absorbed partially at first, now absorbed the agglutinin completely; and, in addition, four strains, M 12, 13, 14, and 17, which had before shown no absorbing power, now effected complete absorption. The remaining strains absorbed slightly.

In seeking an explanation of these anomalous results, it was noted that the suspensions of M 43 used to test the result of absorption in the two last mentioned experiments were not the same as in the first (shown in Experiment 1 of the table), and it appeared possible that it was to this fact that the irregularity in absorption might be attributed. The exact reason of the variability did not suggest itself until it was found that later sub-cultures of M 43 differed from the earlier in absorptive capacity. This discovery at once suggested the following explanation, on the hypothesis that the antigens of M 43 had been modified during sub-culture:

- (1) The original culture contained the two antigens A and B.
- (2) The serum produced with this culture contained the agglutinins corresponding to both A and B.
- (3) After prolonged sub-culture changes took place in two directions, (*a*) some strains losing the A type of antigen, and (*b*) others losing the B type.

The evidence in support of this is:

- (1) The original culture absorbed the agglutinin from an A type of serum (M 1 serum) and also from a B type of serum (M 17 serum).
- (2) From M 43 serum, which agglutinated M 1 and M 17 equally, A agglutinin was removed by M 1 whilst B agglutinin was left behind, and B agglutinin was removed by M 17, whilst A agglutinin was left behind. (See p. 148.)
- (3) One of the later cultures of M 43 absorbed the B agglutinin from M 17 serum, but failed to absorb the A agglutinin from M 1 serum or from M 43 serum.

Another of the later cultures absorbed the A agglutinin from M 1 serum, but failed to absorb the B agglutinin from M 17 serum or from M 43 serum.

In the light of this explanation the results recorded in Table VIII may be interpreted. In the first experiment the culture of M 43 used for testing the absorbed serum contained A antigen alone or in predominance, and consequently registered only the absorption of agglutinin by strains possessing A antigen (the strains M 1, etc.). In the second experiment (Table VIII) the absorbed serum was tested with a culture of M 43, which contained mainly B antigen, and consequently registered only the absorption of agglutinin by organisms possessing B antigen (strains M 17, etc.).

Whether the two components of M 43 were present as separate individuals in the cerebro-spinal fluid of the case, or whether they are descendants from a single coccus has not been determined. There is evidence that one of the daughter strains is either still a mixture or still capable of variation, since one strain which absorbed A agglutinin but not B now absorbs B but not A. It may be assumed therefore that the differences in absorptive capacity between different sub-cultures depend upon the predominance of one or the other component.

The same explanation serves to account for another series of absorption tests, the results of which were at first puzzling. M 43 serum was absorbed with various strains, including M 43, and then tested on M 7, a strain possessing A antigen. It was found that the M 43 strain failed to remove agglutinin for M 7. The reason, as is now evident, was that the particular strain of M 43 which was used for absorption did not contain much A antigen, and therefore behaved like the strains M 12, 13 and 17 (also known to possess little A antigen), and unlike M 5 (known to possess A antigen), which was found to remove the agglutinin for M 7.

In order to complete the classification of the remaining Group I strains the method illustrated in Table IX was used to ascertain by which of the two agglutinins (A or B) in M 43 serum the agglutination of a particular strain containing one of the corresponding antigens was produced. The tests with the strains M 11, 14 and 15 containing only C antigen are inserted as controls. Evidently M 1 contains A antigen and not B antigen, as it removes the agglutinin for M 8, but not the agglutinin for M 59; for the converse reason the strains M 12, 13 and 17 contain B antigen but not A. This method of utilising the A and B components of the agglutinins in M 43 serum has been applied to five other spinal strains of Group I, and has demonstrated that strains M 48, 52 and 53 contain A antigen but not B, and that the strains M 50 and M 56 contain B antigen but not A.

TABLE IX.

M 43 serum absorbed with spinal strains representing three sub-groups of Group I. Serum titrated before and after absorption and tested on M 43 (homologous), M 8 (combines with agglutinin A), M 59 (combines with agglutinin B).

Method. 5 mg. of culture (unheated suspension) per c.c. of 1 in 50 serum.

Absorbing Strain	Test on M 43				Test on M 8				Test on M 59			
	100	400	800	1600	100	400	800	1600	100	400	800	1600
Nil . .	+	+	±	±	+	+	+	-	+	+	+	±
A M 1 .	±	±	±	-	-	-	-	-	+	+	+	±
B { M 12 .	±	±	±	-	+	+	+	-	-	-	-	-
M 13 .	±	±	tr.	-	+	+	±	-	-	-	-	-
M 17 .	±	tr.	-	-	+	+	+	-	-	-	-	-
C { M 11 .	+	+	±	tr.	+	+	+	-	+	+	+	±
M 14 .	+	+	±	±	+	+	±	-	+	+	+	±
M 15 .	+	+	±	±	+	+	+	-	+	+	+	±

Certain other points of interest appear in Table IX with reference to M 43 serum and culture. The test suspension of M 43 seems to contain a proportion of A antigen as well as B, though the latter preponderates.

Although M 43 culture originally absorbed all three agglutinins, the serum produced with M 43 does not contain much C agglutinin. (*See Table I for its action upon M 11, 14 and 15.*) It is possible that immunisation was not carried far enough to develop this agglutinin from the antigen shown by the original absorption test to be present.

M 10 Serum.

M 10 was a Group I strain with very complete antigenic properties. The evidence of this is found in the following observations, which have already been referred to; it absorbed all three agglutinins of Group I and produced a serum which agglutinated all the Group I spinal strains (see Table I). In the preceding section it has been shown that M 43 serum contained two agglutinins, A and B, and that the Group I strains could be divided according as they (1) picked out A agglutinin only; (2) B agglutinin only; or (3) failed to remove either agglutinin. Among the last-mentioned there were seven strains which possessed in addition to the above negative characteristic the capacity to absorb readily the homologous agglutinin from M 15 serum, *i.e.* C agglutinin.

Following the method just described in dealing with M 43 serum, it can be shown (Table X) that these seven strains, excepting M 41, which are not differentiated by simple agglutination tests with M 10 serum from the other two sub-groups, A and B, of Group I, fail to

absorb the homologous agglutinin A from M 10 serum, but remove the agglutinin for M 14, *i.e.* C agglutinin.

M 41 originally gave the same reactions as the other six strains. In the experiments at this stage it had become more agglutinable, but large amounts of culture were required to remove the agglutinin for itself. When this was effected the agglutinin for other members of the sub-group C was also removed.

TABLE X.

M 10 serum absorbed with spinal strains to show the presence of two agglutinins, A and C. Serum titrated before and after absorption and tested on M 10 and M 14.

Method. One tube of culture. 30-40 mg., added to 2.5 c.c. of 1 in 50 serum.

Absorbing Strain	Test on M 10				Test on M 14		
	200	400	800	1600	200	400	800
Nil . . .	+	+	+	+	+	+	±
M 11 . . .	+	+	+		-	-	-
M 14 . . .	+	+	+		-	-	-
M 15 . . .	+	+	±		-	-	-
M 36 . . .	+	+	+		-	-	-
M 41 . . .	+	+	+		+	+	tr.
M 65 . . .	+	+	+		tr.	-	-
M 51 . . .	+	+	+		-	-	-
M 43 . . .	-	-	-		-	-	-
M 10 . . .	-	-	-		-	-	-

TABLE XI.

M 10 serum absorbed with the same spinal strains as in Table IX. Serum titrated before and after absorption and tested on M 15 and M 41 (strains which combine with C agglutinin).

Method. 5 mg. of culture (unheated suspension) per c.c. of 1 in 50 serum.

	Absorbing Strain	Test on M 15					Test on M 41				
		100	200	400	800	1600	100	200	400	800	1600
	Nil . . .	+	+	+	+	±	+	+	+	+	±
A	M 1 . . .	tr.	-	-	-	-	+	+	tr.	-	-
	M 12 . . .	+	+	+	+	tr.	+	+	+	±	tr.
B	M 13 . . .	+	+	+	+	tr.	+	+	+	±	±
	M 17 . . .	+	+	+	+	tr.	+	+	+	+	±
	M 11 . . .	+	+	±	-	-	+	+	±	tr.	-
C	M 14 . . .	+	+	±	-	-	+	+	±	tr.	-
	M 15 . . .	±	±	tr.	-	-	+	+	±	tr.	-

It will be seen from Table XI that while M 1 removes C agglutinin from M 10 serum, the three strains, M 12, 13, and 17, fail to remove this agglutinin. But these three strains have been shown to absorb the homologous agglutinin from M 15 serum, the type serum for C agglutinin, and yet they cannot absorb from M 10 serum an agglutinin with similar combining affinities.

Assuming that in a particular strain the antigens concerned in the absorption of several agglutinins are separable substances, M 10 (combines with A and C agglutinins) and M 17 (combines with B and C agglutinins) may be represented thus in relation to M 15 serum: M 10 (C antigen) absorbs M 15 agglutinin, M 17 (C antigen) absorbs M 15 agglutinin, but M 17 (C antigen) cannot absorb the agglutinin produced by the C antigen of M 10. In explanation it may be conjectured that the antigens of M 10 and M 17, which are alike in their capacity to absorb the agglutinin from M 15 serum, possess differences in structure which are minor but sufficient to constitute a bar to the combination of the agglutinin produced by one with the antigen of the other. Similar instances of such relationship between two antigens have been found, and are reviewed in the section on variability of strains (p. 166). They serve to emphasise the need for caution in taking as a basis for division of strains into types the differences in absorptive capacities in relation to a single serum.

*Comparison between absorptive capacities of Group I and
Group II cerebro-spinal strains.*

Out of the first 46 strains of spinal meningococci, 22, as previously stated, were selected from the results of the agglutination tests for absorption experiments with Group I sera, and all but two, M 16 and M 46, were shown to contain antigens capable of combining with the Group I agglutinins. The remaining 24, all of which were agglutinated by the Group II sera, will now be compared in respect of their capacity for absorbing agglutinin from those sera.

The method of absorption adopted in defining the agglutinins of Group I, that is, the addition of a heated suspension containing 4 mg. of culture per c.c. to an equal volume of 1 : 25 dilution of serum, was not found suited to the absorption of Group II agglutinins, since so little impression was made even with the homologous strain. It was found necessary to use larger amounts of culture.

TABLE XII.

M 23 serum and M 24 serum (both Group II) absorbed with spinal strains of Group II. Each serum titrated and tested before and after absorption on the respective homologous strain.

Method. M 23 serum: 1.5 c.c. of 1 in 25 serum + growth from whole ascitic agar tube, approx. 30 mg. M 24 serum: 3 mg. of culture (heated suspension) per c.c. of 1 in 50 serum.

Absorbing Strain	M 23 Serum Test on M 23				M 24 Serum Test on M 24			
	100	200	400	800	100	200	400	800
Nil . . .	+	+	+	+	+	+	+	±
M 18 . . .	±	±	±	tr.	±	±	tr.	-
M 19 . . .	±	±	±	tr.	+	+	±	tr
M 20 . . .	±	±	tr.	-	±	tr.	tr.	-
M 21 . . .	±	±	±	-	±	±	tr.	-
M 22 . . .	±	±	±	-	±	±	±	tr.
M 23 . . .	±	±	±	±	±	tr.	-	-
M 24 . . .	±	±	±	±	tr.	tr.	-	-
M 25 . . .	±	±	±	tr.	±	tr.	tr.	-
M 26 . . .	±	tr.	-	-	±	±	tr.	tr.
M 27 . . .	±	±	±	tr.	+	±	tr.	-
M 28 . . .	+	±	±	tr.	tr.	tr.	tr.	-
M 29 . . .	tr.	tr.	tr.	-	±	±	tr.	-
M 30 . . .	±	tr.	tr.	-	±	tr	tr.	-
M 31 . . .	+	+	+	+	+	+	+	±
M 32 . . .	+	+	+	+	+	+	+	tr.
M 33 . . .	+	+	+	+	+	+	+	±
M 34 . . .	+	+	±	tr.	+	±	tr.	tr.
M 35 . . .	+	±	±	tr.	±	tr.	tr.	-
M 37 . . .	-	-	-	-	±	tr.	-	-
M 38 . . .	tr.	-	-	-	±	±	tr.	-
M 39 . . .	±	±	tr.	-	±	±	tr.	tr.
M 42 . . .	+	+	±	tr.	+	±	±	tr.
M 44 . . .	±	±	tr.	tr.	±	tr.	tr.	-
M 45 . . .	+	±	tr.	tr.	±	±	tr.	tr.

In Table XII the results with M 23 and M 24 sera are given. The amount of absorption from M 23 serum ranges from slight diminution of agglutination in the highest dilutions in the case of M 35 to complete absorption with M 37. After absorption with certain strains including the homologous, the test suspension was still agglutinated to the same height as before absorption, but agglutination was incomplete in every dilution. Three strains only, M 31, 32, and 33, failed to absorb any of the agglutinin for M 23. A similar result was obtained with M 24

serum, the same three strains again failing to absorb appreciably. Thus 21 out of the 24 strains showed similar but not identical absorbing capacities in relation to the two Group II sera.

Comparing the absorption experiments with Group I sera, it was found that one serum, M 15, was absorbed by all except two of the 22 strains in Group I. But absorption experiments upon sera prepared with other individuals of the 20 strains showed that these could be divided into three sub-groups. Similarly, the Group II strains may be subdivided according to their absorptive capacities for the various Group II sera. M 18, for example, produced a serum from which only a minority of the above strains extracted an appreciable amount of homologous agglutinin.

TABLE XIII.

Four Group II sera absorbed with the same suspensions of nine spinal strains. Each serum titrated before and after absorption and tested on the respective homologous strain.

Method. M 32 serum: 10 mg. of culture per c.c. of 1 in 100. M 18 serum: 10 mg. of culture per c.c. of 1 in 100. M 33 serum: 10 mg. of culture per c.c. of 1 in 100. M 24 serum: 5 mg. of culture per c.c. of 1 in 50.

Absorbing Strain	M 32 Serum Test on M 32				M 18 Serum Test on M 18				M 24 Serum Test on M 24				M 33 Serum Test on M 33			
	200	400	800		200	400	800	1600	100	200	400	800	200	400	800	1600
Nil . . .	+	+	+		+	+	+	+	+	+	+	+	+	+	+	±
M 9 . . .	+	+	±		+	+	+	+	+	+	+	tr.	+	+	tr.	-
M 18 . . .	+	+	±		-	-	-	-	±	±	tr.	-	±	±	tr.	-
M 20 . . .	+	+	±		+	+	+	±	+	+	±	-	+	+	±	tr.
M 21 . . .	+	+	±		+	+	+	±	+	±	tr.	-	+	+	±	tr.
M 24 . . .	+	+	±		+	+	+	±	tr.	-	-	-	±	tr.	tr.	-
M 31 . . .	+	+	±		+	+	+	+	+	±	tr.	-	±	±	tr.	tr.
M 32 . . .	-	-	-		+	+	+	±	+	±	tr.	-	+	±	±	tr.
M 33 . . .	+	+	±		+	+	±	-	+	+	tr.	-	±	tr.	-	-
M 42 . . .	+	+	tr.		+	+	+	±	tr.	-	-	-	+	+	±	tr.

Table XIII shows that there are at least four different Group II agglutinins. Four sera were absorbed, each with the same suspensions of the same strains; the amount of culture used was sufficient or almost sufficient to exhaust the sera in the case of the respective homologous strains. Among those strains not absorbing the homologous agglutinin of M 18 serum is M 24, although M 18 was found to absorb the agglutinin from M 24 serum; this relationship between two strains and their

respective sera has been repeatedly observed. M 31, 32, and 33 are seen to be capable of partially absorbing M 24 serum when added in larger amounts than were used previously (Table XII). Complete absorption of M 18 and M 32 sera was effected only by the homologous strains. M 33 agglutinin was absorbed to some extent by all the strains, including M 9 (Group I), thus connecting the two groups.

TABLE XIV.

M 24 serum (Group II) absorbed with seven spinal strains representing three sub-groups of Group I. Serum titrated before and after absorption and tested on M 18, M 61, and the homologous strain (all Group II).

Method. 10 mg. of culture (unheated suspension) per c.c. of 1 in 50 serum.

Absorbing Strain	Test on M 24				Test on M 18				Test on M 61			
	100	200	400	800	100	200	400	800	100	200	400	800
Nil . . .	+	+	+	±	+	+	+	±	+	+	+	+
M 1 . . .	+	+	±	±	+	+	+	±	+	+	+	±
M 12 . . .	+	+	±	±	+	+	+	±	+	+	+	±
M 13 . . .	+	+	+	±	+	+	±	±	+	+	+	±
M 17 . . .	+	+	±	±	+	+	+	±	+	+	+	±
M 11 . . .	+	+	±	-	+	+	±	tr.	+	+	+	±
M 14 . . .	+	±	±	-	+	+	±	tr.	+	+	+	±
M 15 . . .	+	±	±	-	+	+	±	tr.	+	+	+	tr.

A further link between the two groups is shown in Table XIV. M 24 serum was absorbed, titrated, and tested on three strains of Group II, the dilutions being identical in each case. It is seen that the Group I strains, M 11, 14, and 15 removed from the serum a little of the agglutinin for M 24 and a trace for M 18. With larger amounts of culture the absorption of M 24 agglutinin was increased (Table XV).

TABLE XV.

M 24 serum absorbed with increased quantities of three spinal strains (see Table XIV). Titre of serum before and after absorption for the homologous strain.

Method. Growth from one tube to 2 c.c. of 1 in 50.

Absorbing Strain	Test on M 24			
	100	200	400	800
Nil . . .	+	+	+	±
M 11 . . .	±	tr.	tr.	-
M 14 . . .	±	tr.	tr.	-
M 15 . . .	+	+	tr.	-

Agglutinin absorption with naso-pharyngeal strains.

The preceding experiments on absorption with spinal meningococci have shown that many differences exist between the absorptive capacities of different strains and that for the accurate definition of these a large number of sera is required. This fact, on the one hand, indicates that the difficulty will necessarily be as great, and is likely to be greater, in applying the same methods to the identification of many of the naso-pharyngeal strains with meningococci from the cerebro-spinal fluid of cases of meningitis; and, on the other hand, it emphasises the danger of error in excluding the former from the meningococcus species on the grounds of their failure to absorb agglutinin from a few standard sera. In the light of the data ascertained as to the combining affinities of the agglutinin in a number of spinal meningococcus sera, the present task is to compare the combining capacities of the pharyngeal strains for those agglutinins, or, alternatively, to test the absorbing power of spinal strains upon sera prepared with pharyngeal strains. The spinal Group II agglutinating sera, with which a large number of pharyngeal strains have been shown to react (Table II), will be dealt with in the first place.

Relationship of the naso-pharyngeal strains to the agglutinins of Group II.(1) *Tests with M 24 serum.*

The majority of the pharyngeal strains which were agglutinated by M 24 serum were tested as to their absorptive capacity for the homologous agglutinin. It was found that M 24 itself reduced the agglutinating titre of the serum below 1-100, when added in the proportion of 5 mg. of culture to each c.c. of 1-50 dilution of serum; and this method was adopted in the case of the pharyngeal strains. For the sake of condensation only a few of the results are given in tabular form. These are representative of the whole series and are shown in Table XVI, from which it will be seen that some strains caused complete absorption, some partial, and others were negative. The absorbed dilutions were titrated in duplicate and were tested on M 23 as well as on the homologous strain: the results as a rule agreed.

TABLE XVI.

M 24 serum absorbed with 13 naso-pharyngeal strains. Serum titrated before and after absorption and tested on M 24 and M 23.

Method. 5 mg. of culture (unheated suspension) per c.c. of 1 in 50 serum.

Absorbing Strain	Test on M 24			Test on M 23			
	100	200	400	100	200	400	800
Nil . . .	+	+	+	+	+	+	+
NP 1 . . .	+	+	+	+	+	+	+
NP 3 . . .	+	+	+	+	+	+	+
NP 8 . . .	±	tr.	—	+	+	±	tr.
NP 10 . . .	tr.	—	—	+	+	±	—
NP 47 . . .	±	tr.	tr.	+	±	tr.	—
NP 58 . . .	+	+	+	+	+	+	±
NP 60 . . .	+	+	±	+	+	+	tr.
NP 72 . . .	—	—	—	—	—	—	—
NP 73 . . .	tr.	tr.	—	+	+	±	—
NP 78 . . .	—	—	—	+	+	tr.	—
NP 80 . . .	±	tr.	—	tr.	—	—	—
NP 87 . . .	tr.	tr.	—	—	—	—	—
NP 85 . . .	±	tr.	—	+	+	±	—

The following is a summary of the complete results by this method of absorption:

Total strains tested, 58.

Negative: 10 strains (NP 1, 3, 15, 19, 21, 33, 46, 58, 71 and 86).

Definitely positive, *i.e.* reduction in titre to incomplete at 1:100: 23 strains (NP 7, 8, 9, 10, 28, 34, 35, 37, 38, 45, 47, 50, 62, 72, 73, 74, 78, 80, 83, 85, 87 and 89).

Slight absorption: 25 strains. The absorption with these was generally increased with larger quantities of culture: for example, the whole growth from a glucose ascitic slope added to 1 c.c. of 1-50 completely exhausted the serum in the case of NP 2, 20, 23, 25, 26 and 27. If spinal and pharyngeal strains agglutinated by Group II sera are compared as a whole, there will be found close agreement in respect of their absorbing capacities for the homologous agglutinin of M 24 serum. It was shown that 21 out of 24 agglutinating spinal strains definitely absorbed the agglutinin from this serum, and the remaining three also absorbed when the amount of culture was increased.

(2) Tests with M 18 serum.

This serum agglutinated 32 out of the 86 pharyngeal strains to 1-400 or over. The absorptive capacities of 15 were tested in relation

to this serum. Seven of these, NP 46, 58, 64, 72, 78, 85 and 87, failed to absorb the homologous agglutinin. The results with the eight other strains are shown in Table XVII, in which are included for comparison nine spinal-strains of Group II. The table is arranged in a different manner from any of the others. The first column enumerates the pharyngeal and spinal strains agglutinated by the serum, before absorption, up to 1-800 at least, each suspension being the same throughout the experiment. The rest of the table shows that certain of these strains agglutinated (+) and others failed to agglutinate (—) in a dilution of 1-200 after absorption with the strain at the head of each column. Thus in the second column, showing the results of absorption with NP 10, all except NP 40 failed to agglutinate in 1-200. The amount of culture used for absorbing extracted completely (*i.e.* in the highest concentration of serum tested) the agglutinin for the absorbing strain in every instance excepting NP 35; in a few cases where the results were not quite sharp, a trace of agglutination was taken as negative and a marked though incomplete reaction as positive.

TABLE XVII.

M 18 serum absorbed with spinal and pharyngeal strains.

Method. 10 mg. of culture (unheated suspension) per c.c. of 1 in 100 serum. All the strains in the first column were agglutinated to 1 in 800 at least before absorption. Table shows the agglutination (+ or —) in 1 in 200 dilution after absorption with the strains at the head of each succeeding column after the first.

	NP 10	NP 28	NP 35	NP 54	NP 80	NP 48	NP 73	NP 40	M 64	M 66	M 22	M 33
NP 10	—	+	+	+	—	—	+	+	+	—	—	+
NP 28	—	—	+	—	—	—	+	+	—	+	—	—
NP 35	—	+	+	—	—	—	—	+	+	—	—	+
NP 54	—	—	+	—	+	—	+	+	—	+	—	—
NP 80	—	+	+	—	—	—	—	+	+	—	—	+
NP 48	—	+	+	+	—	—	+	+	+	+	—	+
NP 73	—	—	+	—	—	—	—	+	+	—	—	+
NP 40	+	+	+	—	+	—	+	—	—	—	+	—
M 18	—	+	+	—	—	—	—	+	+	+	—	+
M 27	—	+	+	—	—	—	+	+	+	+	—	+
M 61	—	+	+	—	—	—	+	+	—	+	—	+
M 33	—	—	+	—	—	—	+	+	—	+	—	—
M 26	—	+	+	+	+	—	+	+	+	+	—	+
M 28	—	+	+	+	—	—	—	+	+	—	—	+
M 29	—	+	+	+	—	—	+	+	+	—	—	+
M 37	—	+	+	—	—	—	—	+	+	—	—	+
M 44	—	+	—	—	—	—	—	+	+	—	—	+

The results show that five pharyngeal strains, NP 10, 54, 80, 48 and 73, and one spinal strain, M 22, have removed the agglutinin for themselves and at the same time the homologous agglutinin. They differ in capacity for absorbing agglutinins which act upon strains other than the homologous. This somewhat anomalous result may be due to incomplete exhaustion of the serum and the presence of small quantities of agglutinin still remaining, which influences some strains more strongly than others. The strains can be arranged in a series with diminishing capacity for absorption; NP 48 absorbs the agglutinin for all the strains in the left-hand column, and is followed closely by NP 10 and M 22, while NP 40 removes only the agglutinin for itself.

Certain conclusions are to be drawn from these results. (1) No distinction can be made between the spinal and pharyngeal strains; (2) grouping by this method of absorption is indistinct; and (3) differences in absorptive capacity are revealed.

(3) *M 32 serum and M 33 serum.*

Certain pharyngeal strains agglutinated by the above two sera were identified by absorption tests with the spinal strains which produced these sera.

M 32 serum was absorbed with that amount of culture which sufficed in the case of the homologous strain to remove the agglutinin for itself. With this quantity three spinal strains, M 23, 38 and 66, and three pharyngeal strains, NP 5, 12 and 81 removed completely the homologous agglutinin.

The agglutinin from M 33 serum was completely removed by M 24, NP 62 and NP 39. Under the same conditions certain other spinal and pharyngeal strains reduced the homologous agglutinin from 1-1600 to 1-800 and 1-400.

Summary.

A general survey of these results shows that 33 of the 86 pharyngeal strains have exhausted the homologous agglutinin of one or more of the four Group II spinal sera. No less than 29 other pharyngeal strains, not included in the above 33, were agglutinated up to 1:400, or in even higher dilutions. It has not seemed to me a profitable task, considering the range of variation in absorptive capacity of the spinal strains themselves, to attempt to "place" all these strains. I am of the opinion that the results obtained are sufficient to justify the conclusion that a strain which agglutinates to 1:400 with one or more sera of Group II will be found to absorb agglutinin from some one or other Group II serum.

*Relationship of pharyngeal strains to the agglutinins
of Group I.*

The simple agglutination tests summarised in Tables I and II have shown that the proportion of strains in the pharyngeal series which are agglutinated completely to 1-400 and over by Group I sera is much lower than that in the spinal series, comprising in the latter 27 out of 66 strains, and in the former 8 out of 86. Evidence of affinities in the pharyngeal strains with the spinal antigen of Group I, though not revealed by simple agglutination, has been indicated by the agglutinogenic tests, and confirmation is furnished by the experiments on agglutinin absorption. The results with a number of pharyngeal strains, in which such evidence has been sought, are summarised in Table XVIII. The agglutination reactions of the above strains to the two group sera are contrasted with their capacities to absorb agglutinin from these sera and to produce agglutinin for spinal strains of Group I. For convenience, the associated affinity, when demonstrated, with spinal sera of Group II is included in the table and will be discussed later.

It will be seen from this table that the three pharyngeal strains, NP 1, 53, and 40, were capable of binding one or more of the Group I agglutinins, A, B and C. NP 1 and 53 removed the agglutinins for M 1 and M 15 (*i.e.* A and C agglutinins), and NP 40 removed the agglutinin for M 15 (*i.e.* C agglutinin only). As regards B agglutinin (corresponding to M 17 antigen), no pharyngeal strain is quite equal to M 17 in capacity for absorbing B agglutinin from M 17 serum, but partial though distinct absorption was produced by several strains. The results are given later in detail.

A few pharyngeal strains therefore possess, like all but two of the spinal Group I strains, capacity to absorb one or more of the three Group I agglutinins. The two exceptional spinal strains which failed in this capacity are M 16 and M 46. The question now arises whether there are in the naso-pharyngeal series strains which stand in the same relation as M 16 and M 46 to the majority of the spinal Group I strains, *i.e.* possess antigens related to but not identical with those producing the three agglutinins of Group I. It has been found, as indicated in the table, that these specialised antigens of M 16 and M 46 are represented respectively in two pharyngeal strains, NP 2 and NP 43. The method of identification is described in the following experiments in which each strain is dealt with separately:

TABLE XVIII.

Table showing evidence of serological relationship of naso-pharyngeal strains to spinal strains of Group I, and associated affinity with Group II strains.

Strains	Highest agglutination with sera of		Absorptive and agglutinogenic relationship to Group I	Affinity with Group II
	Group I	Group II		
NP 1	1 : 800	—	M 1 and M 15 sera completely absorbed	
NP 53	1 : 1000	1 : 200	M 1 and M 15 sera completely absorbed	
NP 67	—	1 : 200	M 17 and M 43 sera partially absorbed	
NP 2	1 : 400	1 : 400	M 16 serum completely absorbed	M 24 serum partially absorbed
NP 3	1 : 400	1 : 400	M 17 and M 43 sera partially absorbed	M 33 serum absorbed by NP 4
NP 4	1 : 400	1 : 800		
NP 65	1 : 1000	1 : 800		
NP 41	1 : 400	1 : 400	Produces agglutinin for Group I. NP 41 serum completely absorbed by M 15	Produces agglutinin for Group II
NP 5	1 : 200	1 : 400	M 17 and M 43 sera partially absorbed	M 32 serum completely absorbed: M 24 and 33 sera partially absorbed
NP 40	1 : 100	1 : 1000	Produces agglutinins for Group I. M 15 serum completely absorbed	Produces agglutinins for Group II. M 24 and 33 sera partially absorbed
NP 71	1 : 200	1 : 100	M 17 and M 43 sera partially absorbed	
NP 11	—	1 : 200	Produces agglutinins for Group I. M 17 serum partially absorbed. NP 11 serum completely absorbed by M 17 and M 46	Produces agglutinins for Group II
NP 36	—	1 : 400	Both produce agglutinins for Group I NP 43 serum completely absorbed by M 46	Produce agglutinins for Group II
NP 43	—	1 : 400		
NP 82	—	—	M 43 and M 10 sera partially absorbed	
NP 86	—	—	M 43 serum partially absorbed	
NP 44	—	1 : 100	Produces agglutinins for Group I. NP 44 serum absorbed by M 48, Group I	Produces agglutinins for Group II. M 33 serum partially absorbed

NP 2.

NP 2, which was agglutinated by Group I and Group II sera, absorbed partially the homologous agglutinin both from M 24 and M 33 serum (Group II), but did not remove any of the three agglutinins

of Group I. M 16 serum (titre 1 : 1600), agglutinated NP 2 to 1 : 800. From this serum NP 2 absorbed the homologous agglutinin almost as well as M 16 itself. The only spinal strains which absorbed M 16 agglutinin were M 15 and M 36, and they only reduced the titre from 1 : 1600 to 1 : 400 by a single application of culture.

NP 43.

In Table XIX M 46 is shown to be almost identical with NP 43 in its capacity for absorbing the homologous agglutinin from NP 43 serum. No other single spinal strain tested was found to remove completely the homologous agglutinin, even when added in large amounts and on successive occasions.

TABLE XIX.

NP 43 serum absorbed with twelve spinal strains. Titre of serum before absorption (1 in 1600) and after absorption when tested on the homologous strain.

Method. 10-20 mg. of living culture per c.c. of 1 in 50 serum.

Absorbing Strain	Test on NP 43		
	100	400	800
Nil	+	+	+
M 9	+	+	+
M 12	+	+	+
M 15	+	+	+
M 16	+	+	+
M 17	+	+	+
M 18	+	+	±
M 24	+	+	±
M 32	+	+	+
M 33	+	+	+
M 38	+	+	+
M 46	±	tr.	-
M 64	+	+	+
NP 43	tr.	tr.	-

Relationship of other pharyngeal strains to spinal strains.

NP 11.

NP 11 was nearly two years in culture, during which it underwent some modification. Originally it was agglutinated by Group II sera and not by Group I. A serum prepared with it agglutinated more strongly the spinal strains of Group I than those of Group II. After

a time NP 11 lost its agglutinability to its own serum and a second serum was prepared from it. The second serum was much more quickly produced than the first, and attained a titre of 1-2000. This serum, five times the titre of the first, only agglutinated up to 1-100 those spinal strains of Group I which were agglutinated to the full titre of the first serum. In Table XX is given an absorption experiment with the first serum, the absorbed dilutions being tested on M 10 and M 56 (Group I) in addition to the homologous strain, which at that time was unfortunately only slightly agglutinable. M 46, which was agglutinated completely to 1-400, absorbed the agglutinin as well as NP 11. It is shown also that M 17 absorbed the agglutinin equally well, although NP 11 can only partially absorb M 17 serum; M 46 was unable to effect any absorption from M 17 serum.

TABLE XX.

NP 11 serum absorbed with five spinal strains. Serum titrated before and after absorption and tested on M 10, M 56 and NP 11.

Method. 20 mg. of living culture per c.c. of 1 in 50 serum.

Absorbing Strain	Test on M 10			Test on M 56				Test on NP 11	
	100	200	400	100	200	400	800	100	200
Nil	+	+	+	+	+	+	±	+	±
NP 11	-	-	-	±	-	-	-	-	-
M 17	-	-	-	tr.	-	-	-	tr.	-
M 46	-	-	-	-	-	-	-	-	-
M 9	+	±	tr.	+	±	-	-	±	±
M 14	+	+	tr.	+	+	+	-	±	±
M 15	+	+	tr.	+	±	tr.	-	±	±

NP 5.

NP 5, which absorbs readily the homologous agglutinin of M 32 (Group II), removes partially M 17 agglutinin (agglutinin B of Group I). Complete absorption of the serum was obtained when the dose of culture was increased beyond that required to exhaust M 17 serum with M 17 itself.

NP 41.

NP 41, which was agglutinated to 1-400 by M 10 serum, did not remove from that serum either the agglutinin for M 10 or that for M 15; it failed also to remove the homologous agglutinin from M 15 serum. A serum prepared with NP 41 agglutinated M 15 and NP 41 equally well, the reaction being complete in 1-400, well marked in 1-800. It

was found that M 15 was identical with NP 41 in removing the homologous agglutinin from that serum. A second interesting anomaly was observed in that the six strains, M 1-M 6, could not remove from NP 41 serum the agglutinin for M 15, although they removed from M 15 serum the agglutinin for M 15 (see p. 142).

NP 40.

NP 40 readily removed the homologous agglutinin from M 15 serum, but the spinal strain could not remove the homologous agglutinin from the pharyngeal serum. This relationship is similar to that between NP 41 and M 15, but in the reverse direction.

NP 44.

NP 44 was scarcely agglutinated by any of the spinal sera, but produced a serum which agglutinated to a high titre spinal strains in both groups. The homologous agglutinin of NP 44 serum was absorbed with difficulty, many strains of Group I producing no result. The whole of a plate culture of M 11 added to 2 c.c. of 1 in 50 dilution only reduced the homologous agglutinin from 1-800 to 1-400. Three strains of Group I gave the following result (absorption with 10 mg. of culture per c.c. of 1-100 dilution): M 48 absorbed completely, M 9 was less effective, and M 41 reduced the titre for the homologous strain from 1-800 to 1-400. These strains varied at different times in their capacity for absorbing the serum. The homologous strain itself required to be added in considerable amounts to the serum to produce marked absorption; for example, after absorption with 20 mg. of culture per c.c. of 1-50 dilution, the homologous strain was still agglutinated to the full titre but incompletely in each dilution.

Pharyngeal strains related to M 17 and M 43.

It has been mentioned earlier that a number of pharyngeal strains had a certain combining affinity for agglutinin B in M 17 and M 43 sera. The method of absorption adopted to show the presence of two agglutinins in M 43 serum was followed in the case of certain pharyngeal strains. As shown in Table XXI, M 43 serum was absorbed by five pharyngeal strains and in addition by a number of spinal strains which served as controls. The absorbed serum dilutions were titrated in duplicate and tested on M 43 and M 12. It is shown that M 65, 51, and 64 caused no significant diminution in the agglutinating power of the serum either for M 43 or M 12. M 48 and M 43 absorbed the agglutinin

for M 43 (A agglutinin), and left that for M 12 intact, while M 17 absorbed only M 12 agglutinin (B agglutinin). (The suspension of M 43 used contained the component corresponding to M 1, and therefore registered only the absorption of A agglutinin.) Of the pharyngeal strains, NP 65, 67, and 71 reduced by half B agglutinin, *i.e.* the agglutinin for M 12; the other two pharyngeal strains had practically no effect.

TABLE XXI.

Absorption of M 43 serum with spinal and pharyngeal strains, showing the presence of the two agglutinins A and B. Serum titrated before and after absorption and tested on M 43 and M 12.

Method. 5 mg. of culture (unheated suspension) per c.c. of 1 in 50 serum.

Absorbing Strain	Test on M 43				Test on M 12			
	100	200	400	800	100	200	400	800
Control . . .	+	+	+	±	+	+	+	+
M 51 . . .	+	+	+	tr.	+	+	+	+
M 64 . . .	+	+	+	+	+	+	+	+
M 65 . . .	+	+	+	tr.	+	+	+	±
M 17 . . .	+	+	+	tr.	±	tr.	-	-
M 43 . . .	tr.	tr.	-	-	+	+	+	...
M 48 . . .	±	±	-	-	+	+	+	+
NP 65 . . .	+	+	+	tr.	±	±	tr.	-
NP 67 . . .	+	+	+	tr.	+	+	tr.	-
NP 69 . . .	+	+	+	±	+	+	+	+
NP 71 . . .	+	+	+	tr.	+	±	tr.	-
NP 77 . . .	+	+	±	tr.	+	+	+	±

In addition to the above, other strains which partially removed from M 43 serum the agglutinin for M 17 were NP 5, 52, 82, and 86.

A second experiment with M 43 serum is shown in Table XXI_A. In this example the absorbed dilutions were tested on M 50, a culture which was shown to exhaust M 17 serum. There was distinct absorption with NP 11 and NP 14 (the latter strain was agglutinated to 1-400 by M 43 serum).

The next step was to absorb M 17 serum with the pharyngeal strains which had been shown as above to be related to M 17 through their absorptive capacity for the agglutinin in M 43 serum, which acts upon M 17. It was found that these pharyngeal strains reduced to a varying extent the homologous agglutinin of M 17 serum, but failed to exhaust it completely. Several, however, extracted completely from M 17 serum the agglutinin for M 12 (identical in absorptive capacity with M 17),

TABLE XXIA.

M 43 serum absorbed with five naso-pharyngeal strains. Serum titrated before and after absorption and tested on M 50 (M 50 absorbed M 17 agglutinin).

Method. 2 mg. of culture (heated suspension) per c.c. of 1 in 100 serum.

Absorbing Strain	Test on M 50			
	200	400	800	1600
Nil	+	+	+	±
NP 2	+	+	±	-
NP 4	+	+	+	+
NP 11. . . .	+	±	-	-
NP 14. . . .	+	±	-	-
NP 19. . . .	+	+	+	±
M 43	tr.	-	-	-

and other strains reduced the agglutinin for M 50, a strain similar to M 12.

Briefly stated, the results of experiments of this nature showed that the agglutinins in M 17 serum which acted upon spinal strains related to M 17 in absorptive capacity cannot be a single entity, but must comprise a group of agglutinins differing slightly from each other in combining qualities. The related pharyngeal strains have apparently greater affinity for certain of these agglutinins than for others. This question will be reverted to in the section on variability of serological characteristics.

*Evidence of association of Group I and Group II antigens
in a single strain.*

The agglutination and agglutinogenic tests have shown that both group antigens may be present in a single meningococcus strain, the evidence being most marked in the case of certain less typical spinal strains and in a considerable proportion of the naso-pharyngeal strains. The predominance of one antigen over the other in the typical spinal strains has been generally so definite that cross-absorption tests with their sera have given negative results. The spinal strains of Group I which absorb C but not A or B agglutinin form an exception, since they can remove the agglutinin from M 24 serum (Group II) (see Table XV).

Special attention has been directed to those pharyngeal strains which produce agglutinins for spinal strains of both groups and are sometimes

agglutinated by both group sera. Reference has already been made to certain of these in Table XVIII, and some details of interest will now be considered.

NP 44 serum agglutinated M 18 (Group II) up to 1 : 800. M 24 and M 23 readily removed this agglutinin, and M 9 (Group I) reduced it by half. NP 41 agglutinated M 21 (Group II) and M 15 (Group I) removed this agglutinin as well as that for the homologous strain. NP 43 agglutinated a Group II spinal strain, M 61, to 1 : 800. Two other Group II spinal strains readily absorbed from NP 43 serum the agglutinin for M 61 while only reducing the homologous agglutinin from 1 : 1600 to 1 : 800. M 9 (Group I), on the other hand, absorbed the agglutinin for itself, but did not affect either the agglutinin for M 61 or NP 43.

A further observation of interest in this connection is that a mixture of Group I and Group II strains was often more effective in reducing the homologous agglutinin of one of these pharyngeal sera than either alone. For example, NP 40 serum, which was not completely exhausted by any single spinal strain, was treated with (1) a mixture of Group I suspensions, (2) a mixture of Group II, (3) a mixture of Group I and Group II. The suspensions, the same throughout, were standardised to 4 mg. per c.c., and the serum, diluted to 1 : 50, received exactly 2 mg. of the mixed cocci per c.c. In (1) and (2) the homologous agglutinin was reduced from 1 : 800 to 1 : 400. and in (3) to 1 : 200.

It may be recalled that the parameningococcus of Dopter, obtained at first from the naso-pharynx and later from the cerebro-spinal fluid of cases of meningitis, sometimes produced a serum which agglutinated both parameningococci and meningococci. Experiments quoted in Dr Eastwood's Report¹ showed that parameningococcus serum saturated with meningococci lost its agglutinin for meningococci but retained its agglutinin for parameningococci; while the same serum, saturated with parameningococci, lost its agglutinin for parameningococci but retained its agglutinin for meningococci. If Group I is substituted for meningococcus and Group II for parameningococcus, Dopter and Pauron's results resemble those mentioned with the naso-pharyngeal serum, NP 43. In reports by Ellis and Arkwright² the parameningococcus was identified with their Group II, but Gordon³ could not establish its identity with any of his types of meningococci. Dopter and Pauron divided their

¹ *Journ. of Hygiene*, xv, p. 432.

² *Brit. Med. Journ.*, December 18th, 1915.

³ Gordon and Murray (1915), *Journ. Royal Army Med. Corps*, xxv.

seven strains of parameningococci into three groups by means of saturation tests, and they suggested that perhaps additional varieties might subsequently be found. I am of the opinion that the strains of parameningococci which Dopter found to produce agglutinins for both varieties are like the pharyngeal strains which are agglutinated by Group II sera and produce agglutinins for both Group I and Group II. They differ from the commoner examples of cerebro-spinal meningococci in being less well-defined in relation to the two main groups.

Variability of strains.

In the course of this investigation some instances of variation have been observed which are of importance in their bearing on the position occupied by the less typical strains in the whole group of meningococci. For the most part, excepting variations in agglutinability and minor alterations in absorptive capacity, the various strains under observation have retained in culture their particular serological characteristics. This is not necessarily proof that they remain unvaried, since the method by which the cultures are maintained, *i.e.* by the transference of considerable quantities from one tube to another, may not bring to light possible modifications. As it is not to be expected that the culture would vary *en masse*, variations of individual cocci might occur but be overgrown by the predominant organism. Consequently their demonstration demands that individual cocci should be isolated from the general mass of the culture.

An experiment with that object has been made with M 17 culture. This particular strain was chosen because a change was observed in its absorptive capacity towards its own serum. Originally 2 mg. of culture per c.c. of 1-50 serum was sufficient to reduce the agglutinating power of the serum below 1:100, but later it was necessary to use much larger amounts to bring about the same result. I therefore endeavoured to ascertain whether all the individual cocci in the culture were identical, the idea having suggested itself that the presence of some with diminished absorptive capacity might account for the necessity of using increased quantities of culture to effect absorption. M 17 was plated and 40 single colonies were grown in subculture. Each of these was used separately to exhaust M 17 serum, and one strain only was found which failed to absorb the agglutinin for the whole culture while removing the agglutinin for itself. The other 39 strains removed the agglutinin for themselves and for the whole culture. The aberrant strain, after remaining in subculture for some time, was again plated.

Four single colony strains were grown; three removed the whole agglutinin and one failed. Either the single colony first selected was not from a single organism or a certain number had reverted to the predominant type. The last non-absorbing strain, designated M 17 K, was subcultured twice and again plated. Twelve single colonies were grown in subculture.

TABLE XXII.

Agglutination and absorption test of M 17 serum with M 17 (whole culture) and twelve single-colony strains of M 17. Serum before absorption titrated and tested on each strain, and after absorption again on each strain and on the whole culture.

Method. Three successive additions to 2 c.c. of 1 in 50 serum: (1) 5-10 mg. of each culture, (2) growth from whole tube two hours later; after centrifuging the clear fluid was pipetted off and (3) growth from a whole tube was again added.

	Before absorption				After absorption							
	v. absorbing strain				v. M 17 whole culture					v. absorbing strain		
	200	400	800	1600	100	200	400	800	1600	100	200	
M 17 (whole culture)	.	+	+	+	-	-	-	-	-	-	-	-
M 17 A	.	.	.	+	+	+	+	tr.	-	-	-	-
M 17 B	.	.	.	+	+	+	+	+	-	-	-	-
M 17 C	.	.	.	+	+	+	+	tr.	-	-	-	-
M 17 D	.	.	.	+	+	+	+	tr.	-	-	-	-
M 17 E	.	.	.	+	+	+	+	±	-	-	-	-
M 17 F	.	.	.	+	+	+	+	tr.	-	-	-	-
M 17 G	.	.	.	+	+	+	+	tr.	-	-	-	-
M 17 H	.	.	.	+	+	+	+	±	-	-	-	-
M 17 I	.	.	.	+	+	+	+	tr.	tr.	-	-	-
M 17 J	.	.	.	+	+	+	+	±	-	tr.	-	-
M 17 L	.	.	.	+	+	+	+	tr.	tr.	-	-	-
M 17 M	.	.	.	+	+	+	+	±	-	-	-	-

Table XXII gives the agglutination results with each of these 12 single-colony strains, and demonstrates the absorptive capacity for their own and the homologous agglutinins in M 17 serum. It will be seen that the severe method of absorption adopted ensures the elimination of mere quantitative differences in absorptive capacity. The whole culture absorbed all the agglutinin for itself, but the single-colony strains, while absorbing completely their own agglutinins, only reduced in some cases very slightly the agglutinin for the whole culture. In

simple agglutination the single-colony strains generally reached half the titre of M 17 serum for the whole culture. In this respect they resembled some of the pharyngeal strains previously mentioned which reduced the homologous agglutinin of M 17 serum but failed to exhaust it.

An absorption experiment was carried out with the object of ascertaining whether the agglutinin for the variant of M 17 could be more readily removed from M 17 serum by these pharyngeal strains than the whole agglutinin. In Table XXIII M 17 serum was absorbed with six pharyngeal strains: three spinal strains which do not absorb B agglutinin were included as controls. It will be seen that the agglutinin for the variant, M 17 K, is more readily absorbed by five pharyngeal strains than the whole agglutinin. NP 2, which was shown to absorb M 16 serum, and the three spinal strains had practically no effect. The test suspension of M 17 K was composed of a mixture of the 12 daughter strains (Table XXII) and did not agglutinate as well as most of the individual cultures.

TABLE XXIII.

M 17 serum absorbed with spinal and pharyngeal strains. Serum titrated before and after absorption and tested on M 17 (whole culture) and on M 17 K (variant).

Method. Equal parts of 20 mg. suspensions and 1 in 50 serum.

Absorbing Strain	Test on M 17				Test on M 17 K		
	200	400	800	1600	200	400	800
Nil . . .	+	+	+	+	+	+	tr.
NP 2 . . .	+	+	+	±	+	+	tr.
NP 3 . . .	+	+	+	+	-	-	-
NP 5 . . .	+	+	+	+	-	-	-
NP 14. . .	+	+	±	tr.	tr.	-	-
NP 67. . .	+	±	±	-	-	-	-
NP 86. . .	+	+	tr.	-	tr.	-	-
M 9 . . .	+	+	±	-	+	+	-
M 48 . . .	+	+	+	tr.	+	+	-
M 42 . . .	+	+	+	±	+	+	tr.

In Table XXIV the culture used to test the result of absorption was from M 17 K before the last plating, and agglutinated better than the whole culture. Certain pharyngeal strains have exhausted the serum of the agglutinin for the variant but not for the whole culture. In addition, two strains, NP 5 and NP 65, have reduced the agglutinin for the whole culture to below 1-200. This shows the effect of repeated additions of

culture in increasing the amount of absorption. Much larger quantities of these two cultures were less effective in a single or even in two applications.

TABLE XXIV.

M 17 serum absorbed with nine naso-pharyngeal strains. Serum titrated before and after absorption and tested on M 17 (whole culture) and on M 17 K (variant).

Method. Unheated suspensions of 20 mg. per c.c. added to 1 in 10 serum at intervals until final proportions were 19 mg. of culture per c.c. of 1 in 100 serum.

Absorbing Strain	Test on M 17 (whole culture)			Test on M 17 K		
	200	400	800	200	400	800
Nil . . .	+	+	tr.	+	+	+
M 17 . . .	-	-	-	-	-	-
NP 4 . . .	+	tr.	-	-	-	-
NP 5 . . .	-	-	-	-	-	-
NP 11. . .	+	±	-	tr.	-	-
NP 17. . .	+	tr.	-	-	-	-
NP 65. . .	tr.	-	-	-	-	-
NP 67. . .	+	tr.	-	-	-	-
NP 71. . .	+	tr.	-	-	-	-
NP 77. . .	+	±	-	+	+	tr.
NP 86. . .	+	±	-	-	-	-

To sum up, these results show that the variant of the strain M 17 shows the same capacity for absorbing an agglutinin in M 17 serum as do the pharyngeal strains, NP 3, 4, 5, 11, 17, 65, 67, 71 and 86, and that this agglutinin is different from the predominating agglutinin for the original M 17 since these pharyngeal strains do not remove the latter.

The following variations were observed incidentally in other strains.

A strain of M 6, already referred to, was obtained which had completely lost its early features, namely, the agglutinability towards M 1 serum and the capacity to absorb the agglutinin. On returning to earlier stock cultures on egg, it was found that these had undergone no change. As this is an isolated example of so marked an alteration, I merely record it.

M 16 at first absorbed partially (Group I sera (A agglutinin). Later, as shown, it failed to absorb.

M 43, as related in another place, was resolved into two components; when combined these exhausted the serum produced by the whole culture, though each separately removed only a portion of the agglutinin.

M 12 showed a marked quantitative alteration in absorptive capacity

towards M 17 serum. At first 2 mg. of culture in the form of a heated suspension exhausted 1 c.c. of 1-50 dilution. Later two and a half tubes of fresh culture were added to 4 c.c. of 1-50, and the absorbed serum was tested on M 12, the absorbing strain, and on M 17, the homologous. The reduction for the latter was from 1-800 to 1-400. To the 3 c.c. of serum remaining two more tubes of M 12 culture were added; agglutination of M 12 still occurred. After this second absorption the test on the absorbing strain and on the homologous culture showed that the serum was exhausted. The change in quantity of culture required for absorption of agglutinin for M 12 itself may be explained by the culture being hypersensitive, although it did not agglutinate with normal rabbit serum in 1-25.

M 41 became towards M 10 serum similarly hypersensitive though not auto-agglutinating, and a large quantity of culture was required to remove the agglutinin for itself.

The above alterations in absorptive capacity of M 12 and M 41 recall the alteration in M 17 which led to the discovery of a variant differing qualitatively in absorptive capacity from the whole culture. Reference has been made in a previous part of this report to the possibility of slight differences in structure between two antigens so influencing the combining qualities of the agglutinins they produce as to lead to an apparently great divergence in the serological reactions of the strains containing them. There is little doubt that if a serum had been prepared with the variant of M 17 the whole culture of M 17 would have absorbed the homologous agglutinin, in consequence of the presence therein of the variant. In that case the relationship, for example, of NP 40 to M 15 would have been reproduced. That is to say, NP 40 (cf. M 17) would absorb the homologous agglutinin of M 15 (cf. M 17 K) serum, but M 15 (cf. M 17 K) would be unable to absorb the homologous agglutinin of NP 40 (cf. M 17) serum.

A few of the many examples of this relationship between two strains may be recapitulated:

M 15 absorbed NP 41 serum, but NP 41 could not completely absorb M 15 serum.

M 17 absorbed NP 11 serum, but NP 11 could not completely absorb M 17 serum.

M 10 absorbed M 15 serum, but M 15 could not completely absorb M 10 serum.

M 43 absorbed M 17 serum, but M 17 could not completely absorb M 43 serum.

These observations were referred to in suggesting an explanation for an anomalous result in connection with the absorption of the three agglutinins of Group I (pp. 149-50). It was found that M 10 and M 12 absorbed the homologous agglutinin from M 15 serum, *i.e.* C agglutinin, but M 12 could not absorb the agglutinin contained in M 10 serum, which agglutinated M 15. Those portions of the antigenic substances in M 10 and M 12 which combine with the agglutinin for M 15 may be assumed to stand in the same relation to each other as the antigens in M 17 and M 17 K.

SEROLOGICAL CHARACTERS OF SPINAL STRAINS IN RELATION TO AGE OF PATIENT.

Information concerning the ages of the meningitis patients, from whom the 66 spinal strains under investigation have been obtained, was available in the case of 55 strains.

		Total	Group I	Group II
Patients under 12 years	...	38	15	23
Patients over 12 years	...	17	10	7

In the 15 Group I strains from children under 12 years are included the two aberrant strains, M 16 and M 46.

Although the data are insufficient for any general conclusion to be drawn from them, there is evidently a definite preponderance of Group II strains over the commoner varieties of Group I in a series of strains obtained from meningitis in children under 12 years. In patients over 12, on the other hand, the Group I strains are found rather more frequently.

The disproportion is more marked in infants: of 14 cases under 1 year (included in the above 38) 10 yielded Group II strains and 4 Group I (including one aberrant strain). A similar though even greater preponderance of Group II was found in the naso-pharyngeal series of meningococci from persons of all ages. Thus the meningococci found in the cerebro-spinal fluid of meningitis in the most susceptible individuals tend to approximate in character to those in the naso-pharynx of the general population.

CLASSIFICATION OF MENINGOCOCCI BY SEROLOGICAL TESTS.

This investigation of cerebro-spinal and naso-pharyngeal meningococci has shown that there is no group of naso-pharyngeal cocci which, though identical morphologically and culturally with meningococci, possesses specifically distinct serological characters.

Considering the results of the tests upon the whole series, it has been found that agglutinating sera prepared with certain spinal strains divide the greater part of the series into two groups, the remaining strains being less well defined, both in agglutinability and absorptive capacity, in relation to these two groups. The strains which fail to conform to the division into groups are more frequently of naso-pharyngeal than of cerebro-spinal origin.

On examining my results in relation to the age of the patients, I find that meningococcal strains obtained from young subjects, particularly infants, but also in the case of older children, resemble the naso-pharyngeal strains of non-contacts in two respects: (1) they yield a higher proportion which is not amenable to group differentiation than do the strains obtained from the disease in adults; (2) they also yield a higher proportion of Group II strains compared with adults. It is quite possible that the more virulent meningococci, *i.e.* the strains usually found responsible for the disease in the older and less susceptible elements of the population, are those which conform fully to the two main groups in serological characters, and that divergence from these characters is correlated with less virulence, *i.e.* feebleness for invading the system from the naso-pharynx.

But apart from the above broad distinction there has not been shown any possibility of estimating degrees of virulence from serological reactions, and the question is complicated by the differences in individual susceptibility.

It has been found by Gordon, who obtained his material mainly from the military population, that the serological characters of his spinal strains showed uniformity of type. This difference in the source of material may, to some extent, explain why his results do not agree with mine. The cerebro-spinal strains studied in my report have been obtained almost entirely from the civil population and include a large proportion from cases of meningitis in children.

Two instances given in the present report of cultures obtained from post-basal meningitis in infants may be mentioned in illustration. They showed as marked divergence in serological characters from the two main groups as any strain from the naso-pharyngeal series. Both strains agglutinated slightly with sera of Group II; one, M 46, produced a serum which agglutinated mainly Group I strains and the other, M 55, a serum which influenced only itself to any extent. I hope to show later the position in relation to the two main groups occupied by these strains which differ serologically from the commoner and presumably more virulent spinal strains.

In this connection, as showing that the possibility of these differences existing had been recognised, the Report of the Special Advisory Committee¹ may be quoted.

¹ *Medical Research Committee. Report upon Bacteriological Studies of Cerebro-spinal Fever during the Epidemic of 1915.*

"It must be observed, however, that the strains studied have been epidemic strains from the meninges. It is possible that the meningococci found in the posterior basic meningitis of infants in non-epidemic times will prove to belong to other types and the same seems likely to be true of many of the meningococci found in carriers." The Medical Research Committee's Report says further, with regard to the influence of age on the incidence of the disease: "It is well known that the sporadic form of cerebro-spinal fever as seen amongst the civilian population is mainly a disease of infancy and early childhood. It becomes rarer in adolescence and is uncommon in adults."

The above expression of opinion was foreshadowed by Arkwright¹ in 1909, as the following quotation shows. "One feature of the group of meningococci from sporadic cases taken as a whole appears to be that its members are more frequently found to deviate from the type to which most strains conform than is the case with the epidemic group."

Differences in susceptibility may account for the discovery of strains from meningitis in adults which would be classed on the above principles amongst the least virulent. Such strains however have been relatively uncommon, and it is evident that criteria for the identification of the meningococcus which are based on the serological reactions of meningococci occurring most frequently in adults may exclude strains capable of causing meningitis in children.

The adoption of excessively rigorous standards, which can perhaps be justified as a measure of control in the early stages of an outbreak of cerebro-spinal fever, may explain why Gordon and his fellow-workers² have classified the Gram-negative cocci of the naso-pharynx in a way which I am unable to regard as correct.

The general consensus of opinion is in agreement with the above statement that the meningococci concerned in the recent prevalence of cerebro-spinal fever show a well-marked differentiation by simple agglutination tests into two main groups, a variable number remaining which cannot definitely be placed.

Gordon, however, claims to demonstrate four specific types by means of absorption tests, but differences in absorptive capacity, however well marked, cannot in themselves be taken to be proof of specific differences. I have been able to show that two strains from the same culture may exhibit differences in absorptive capacity which would, according to Gordon's method of classification, resolve them into separate species. Gordon indeed recognises an affinity between his Types 1 and 3, and more recently between Types 2 and 4 also, and in his final conclusion states that it must not be assumed that the limit of their individual variation has yet been defined. Possibly he will discover a still closer relationship in the future. In one of his earlier

¹ *Journ. of Hygiene*, ix.

² Bacteriological Studies, etc. *Medical Research Committee, Special Report Series*, No. 3.

reports¹ he mentions that a strain absorbed the specific agglutinin from sera of Types 1 and 3 and was therefore labelled amphoteric. It seems to me impossible to justify the specific differentiation of two varieties thus linked together.

Reference should also be made to the work of Colebrook and Tanner² who have discussed the carrier problem and have come to the conclusion that most carrier strains are true meningococci. In considering their reasons for adopting this view they discuss two hypotheses to account for the apparent inability of the majority of such strains to determine general infections: (1) most carriers and normal persons possess an adequate natural immunity against the meningococcus, (2) the pharyngeal coccus of the carrier is an attenuated strain incapable of determining a meningitis. While not coming to any final conclusion as to which of the alternative hypotheses is preferable, they apparently consider attenuation of virulence to be an important factor.

A study of the agglutinogenic properties of these irregular and apparently less virulent strains has shown that the sera prepared from certain of them pick out other members of the class in an irregular fashion, and that therefore these strains are not all identical in antigenic capacities. Such sera agglutinate also the presumably more virulent strains which conform to the reactions of the two main groups, thus demonstrating that the strains producing them are antigenically related to the latter. They agglutinate however strains in both groups more or less equally, a result which apparently invalidates the grouping obtained with the standard spinal sera.

Evidently therefore there is some difference between the Group I and II antigens contained in the typical strains and the related antigens in the less well defined strains, *e.g.* the latter may not be affected by an antibody in the sera of the former but nevertheless produce antibodies which have a marked action upon corresponding antigens in the former.

Analogous differences, not revealed by simple agglutination tests, have been demonstrated between the antigens of the typical Group I strains and also between those of Group II. For example, two antigens may combine with, *i.e.* absorb, the same agglutinin though one antigen does not combine with the agglutinin produced by the other.

When it is recalled that variations in antigenic capacity of a similar nature may occur in a strain during sub-cultivation, it is reasonable to assume that the meningococcus antigens are not precisely fixed or stable substances, but are liable to modification under the influence of environment.

I regard therefore these cerebro-spinal and naso-pharyngeal strains which I have investigated as forming a single species, the meningococcus

¹ Identification of the Meningococcus. *Journ. Royal Army Med. Corps*, October, 1915.

² *Journ. Royal Army Med. Corps*, January, 1916.

species, which includes two well defined serological-races linked together by less highly differentiated strains. This tendency for a bacterial species to differentiate itself into races may be regarded as a continuation of the same evolutionary process whereby bacteria with the characteristics of a common genus exhibit emergence into a greater or smaller number of species.

The views expressed by Andrewes and Horder (*Lancet*, 1906, II 711), on the classification of organisms into different species may be usefully applied to racial differences between different strains of meningococci.

"When an arbitrary set of characters is taken as a basis for the classification of a group of natural objects the same phenomena are usually seen—large groups of like objects connected by small groups which differ from them in one or two particulars. If the numerical frequency of each individual like group is represented by the proportional height of a vertical line and the lines are arranged in series, the commoner types stand out boldly above the rarer ones. Only in nature they are plotted out, not in linear series, but in space of two dimensions, as it were, so that the common types stand out as mountain tops above their fellows, each mountain connected by valleys of intermediate types with many of its neighbours. If now the mountains were cut off by a horizontal plane half-way up their sides and attention were paid only to the mountain tops, disregarding the valleys, we should have the popular conception of species."

Similarly in the two series of meningococcal strains the two serological groups stand out above the mass of undifferentiated strains.

As mentioned above, these two groups are not fixed types but may be further sub-divided by means of absorption tests into sub-groups which are probably "centres of variation" in the different stages of evolution of the meningococcus antigens. Such variations in the serological properties of antigens must ultimately depend upon changes in their chemical constitution.

Andrewes (1913), in his Presidential Address, *The Nature and Degree of Specific Differences amongst Bacteria*¹, discussing the importance of chemical evolution in determining the formation of bacterial species, says: "Only now do we begin to get a hint of what I believe to have formed a large part of the course of evolution within the morphological boundaries of bacterial genera—namely, that it has been a chemical rather than a structural one—a change not so much in the configuration of the organism as in that of the protein molecules which build it up." (This conception can, I think, be applied to the evolution of racial types within a species just as much as to the evolution of different species.) "But just as in the building up of higher organisms many proteins are concerned so, though perhaps in a lesser degree of complexity, must the bacterial body be built up of several kinds of protein, in varying combination, some perhaps peculiar to the species others common to many."

¹ *Proc. Royal Soc. Medicine*, VII. (Pathological Section).

And with reference to racial differences of meningococci the Medical Research Committee's Report says: "The races are defined by methods the relative values of which may still be disputed and the differences brought out by these methods may be conjectured to depend upon slight changes in the atom groupings of the bacterial protein determining its reaction with a particular antibody."

Although the validity of the sub-division of the two main groups of meningococci into types upon the results of absorption tests is open to question, there is no doubt as to the importance of such serological tests in furnishing information on the relationship of antigens in different strains. The possibility of the differences in antigenic capacity being correlated with differences in virulence makes their detailed study of more than academic interest.

My observations on the serological and biochemical reactions of the meningococcus have brought me to the conclusion that the meningococcus antigen is composed of two different proteins and it is the relative proportions of these in one strain which decide its position in relation to the two main groups. The further division into sub-groups and atypical strains must on this hypothesis be due to alterations in the configuration of the two specific proteins.

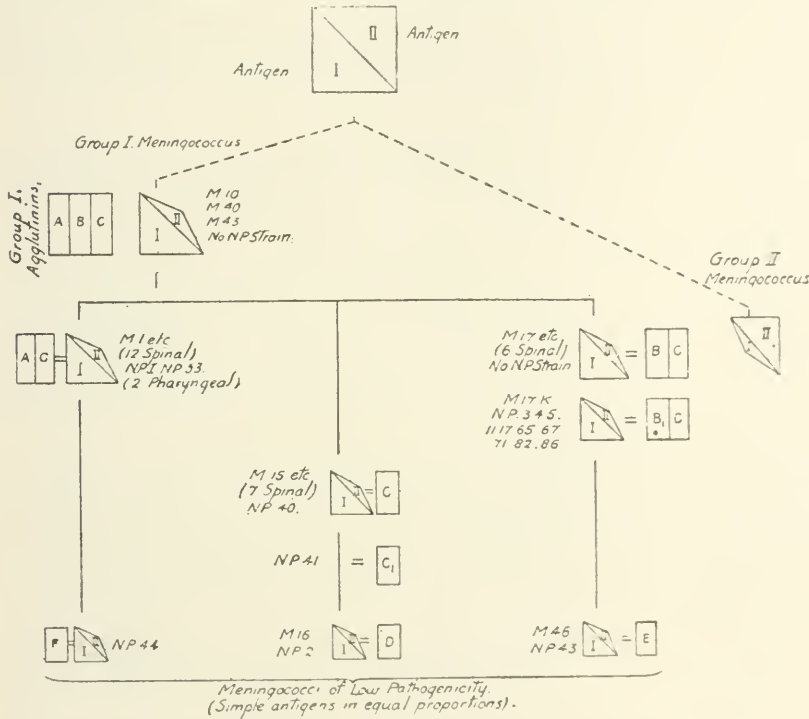
It is probable that the changes in the atom groupings are not haphazard but follow a definite tendency. My results seem to show that in the case of the meningococcus antigens they are in the direction of increased or diminished complexity of structure of the receptor apparatus, with corresponding increase or diminution in the range of combining capacity. This inference is based upon a comparison of the absorptive capacities of strains for the agglutinins contained in different sera.

In the annexed scheme, which is intended to represent graphically evolution of antigenic complexity, I have commenced with a hypothetical complete meningococcus antigen possessing the full antigenic capacities of Group I and Group II.

The actual existence of such strains has not been demonstrated but is at least suggested by the following considerations. In the severe epidemic of cerebro-spinal fever in Silesia during the winter of 1904-1905, the strains of meningococci tested by Lingelsheim all reacted with monovalent agglutinating sera prepared with one of them. The majority of the strains were cultures from the naso-pharynx. It may be argued that Lingelsheim was dealing with only one of the two groups. Against this are his observations that all his strains produced strong and equal fermentation with dextrose and maltose contained in his solid media. There is certainly a possibility, though at the present day no final conclusion can be reached, that his strains each possessed the complete antigenic properties of both groups. It is of interest to note that in subsequent German investigations during the years 1906-1909 (see Dr

Eastwood's Report¹) cerebro-spinal strains were found which exhibited less uniformity in agglutination and fermentation reactions; also aberrant strains were found in the naso-pharynx. It is possible that strains with double antigenic properties such as Lingelsheim may have been dealing with were relatively unstable and capable of being easily resolved into two less complex components with one or other antigen in predominance. An observation by Dr Scott may be quoted in this connection; he obtained from a single culture two strains, one with Group I characters and the other with Group II.

Epidemic Meningococcus (complex antigens in equal proportions).



In their bearing on this question the results of Walker Hall and Peters² are also suggestive. They adopted the plan of comparing the serological reactions of meningococci obtained from the same patient on successive days. In two cases of severe cerebro-spinal fever they found differences. The second of these, recorded at the end of their report, may be quoted as an example. The meningococcus recovered from the cerebro-spinal fluid on the third day absorbed Type 2 agglutinins, while

¹ *Journ. of Hygiene*, xv. 405.

² Changes in the Agglutinability, Fermentation Reactions and Absorptive Capacities of the Meningococcus during the Acute Attack. *Journ. Royal Army Med. Corps*, October, 1916.

that recovered on the fourth day absorbed agglutinins of both Types 1 and 2. It might be suggested that the injection of Dopter's serum after the first withdrawal of fluid had some influence in producing this apparent alteration. Assuming the presence of two components in the spinal fluid, Dopter's serum would reduce the Type 2 cocci and there would be increased probability of isolating a strain containing Type 1 cocci. The work of these authors is of particular interest to me in furnishing as it does some experimental evidence of a virulent strain with double antigenic properties, and I agree with them that "some aspects of the problems associated with the grouping of meningococci may be solved by further study on similar lines."

But, though possibly with rare exceptions, the antigens of the strains found in the present epidemic were less complex and commonly showed a preponderance of I or of II; these are figured in the scheme as descendants of the primary antigens.

A particular study was made of spinal strains containing Group I antigen and related strains from the naso-pharynx, and I have attempted to represent in the remainder of the scheme the plan upon which these various strains appear to be linked together.

Following the genealogical table downwards, a triangle represents the antigen and an oblong the agglutinin with which it is capable of combining, and diminution in complexity of these substances is indicated by decrease in size of the figures. Thus the three most complex antigens of Group I are contained in M 10, 40 and 43, which can bind all three agglutinins A, B and C of Group I; no strain of equal complexity was found in the naso-pharynx. One of these complex strains, M 43, was resolved into two daughter strains, equivalent respectively in serological characteristics to M 1 and M 17; each of them was capable of binding two agglutinins but neither was of sufficient antigenic complexity to bind all three.

Comparing spinal with pharyngeal strains, two pharyngeal strains were found which absorbed the two agglutinins A and C, but none was quite equal to M 17 in absorbing B and C agglutinins. There were seven spinal strains and one pharyngeal strain of still simpler antigenic structure which combined only with C agglutinin.

According to Andrewes and Horder's simile, these three sub-groups of Group I, of which M 1, 15 and 17 are types, would stand out as mountain tops united in the valleys by the undifferentiated spinal and pharyngeal strains such as M 16 and 46, and NP 2, 43 and 44, which can only with difficulty be identified with any other strain. These intermediate types in the valleys, to use their words, illustrate variation and the connection between allied species (here serological races).

The analysis of Group I antigen, and the demonstration of its association with Group II, serve to explain (*a*) different range of valency of different Group I sera and (*b*) action of naso-pharyngeal sera on cerebro-spinal strains, in the following way.

I have represented in the scheme every strain as containing a small portion of Group II antigen in addition to the main antigen of Group I. It will be seen that in the undifferentiated strains the two antigenic substances become proportionately equal and the Group II portion has become of greater relative importance than in the more complex strains. This relationship is reflected in their antigenic capacities, since many produce sera which agglutinate more or less equally strains in the two well defined groups. These latter strains, although they may be well agglutinated by such naso-pharyngeal sera, do not readily absorb the homologous agglutinins, and it has been observed that a mixture of strains of the two groups may be more efficacious in absorbing than strains of either group alone. In the case of the more complex strains on the other hand, *e.g.* M 10 and M 43, the Group II antigen is quantitatively of slight importance, and in the process of immunisation the antibodies are produced against the most prominent antigen. Thus one obtains a serum the agglutinating action of which is largely confined to those strains containing mainly Group I antigen.

In regard to the production of acid in the presence of glucose and maltose it has been found that strains which produce agglutinins mainly of one group generally produce more acid with one sugar than with the other; the fermentation of glucose is associated with the formation of Group II agglutinins, and the fermentation of maltose with the agglutinins of Group I. On the other hand the atypical strains, which have been assumed to contain a simple form of each antigen in about the same relative proportions, often ferment both sugars equally. It has been observed that the latter strains adapt themselves with greater difficulty than the typical strains to growing on glucose agar without added serum.

Following the genealogical table from below upwards and adapting the above considerations to Andrewes and Horder's hypothesis, the sub-groups of Group I, as well as the less well defined Group II, would be types emerging from a mass of undifferentiated forms, and they would owe their greater frequency of occurrence in cerebro-spinal fluid to being more fitted for invasion by chemical adaptation. This adaptation is represented as due to progressive increase in complexity of structure and I have indicated that this progression may be continued until a strain is evolved which is equally well equipped with the complete Group I and Group II receptors. This I have shown by figuring a

hypothetical strain in which both the meningococcus antigens are in equal proportions and of maximum complexity. The introduction into the community, from within or without, of a strain approximating to such a prototype might explain the origin of an epidemic of cerebro-spinal fever.

An epidemic meningococcus of such high complexity would not be stable but would undergo degeneration with rapidity proportional to the resistance it met and would retrace its steps along the path of evolution, giving rise on the way to the two main groups and to the various sub-groups of diminishing complexity. It would end again where it arose in the mass of undifferentiated forms with relatively slight capacity for invading the body from their habitat in the naso-pharynx.

There are thus two conceptions, (1) the slow upward development of complex virulent types, (2) the more rapid devolution or degeneration into simple less virulent types. Both of these processes are likely to take place in the course of an epidemic and may to some extent go on simultaneously.

It will be of interest to examine the evidence available of the serological characters of the meningococci obtained in the early period of the epidemic on Salisbury Plain. Ellis (1915)¹ found his Type 1 eight times and Type 2 three times. Arkwright (1915)¹ reported on nine cases from the First Canadian Contingent on Salisbury Plain; five belonged to the "meningococcus" group and two to the "parameningococcus" group (the former corresponds to Type 1 of Ellis and Group I of this report); one, Chandler, agglutinated slightly with sera of both groups and the remaining strain, Chase, fell in different groups on two occasions. Arkwright concluded that Chandler probably belonged to his meningococcus group and that Chase perhaps contained individual cocci which reacted with different sera. Gordon (1915)², working with 32 strains from various sources, classified 23 in Types 1 and 3 (apparently related in simple agglutinability and probably corresponding to Group I of this report) and 8 in Type 2, the remaining one not conforming to any of the three types.

It is clear that the two serological races were already well defined early in 1915, and there seems little doubt that strains containing Group I antigen were most conspicuous at the commencement of the epidemic. If the results recorded in this report, which deals with strains collected at a later stage of the outbreak, are contrasted with the above, it will be seen that meningococci of Group II have been obtained from the cerebro-spinal fluid rather more frequently than those of Group I. The various strains investigated were obtained from different parts of the

¹ *Brit. Med. Journ.*, II. 881 and 885.

² Gordon and Murray. *Journ. Royal Army Med. Corps*, xxv. 411.

country and their collection was not begun until towards the end of March, 1915.

My strains include many from cases of meningitis in children, and the results are therefore not strictly comparable with those quoted above. The experience of the workers for the military authorities has however been similar, as the following quotation from Lieutenant-Colonel Gordon's report¹ shows: "The meningococci coming from military cases during 1915 were chiefly specimens of type 1 at first, but as the outbreak progressed, type 2 became more abundant. When the disease declined, during the summer of 1915, several specimens of type 3 were met with; and the solitary specimen of type 4 also dated from the later stages of this 1915 outbreak. It was noteworthy that when the disease returned in the last months of 1915, first of all type 1 reappeared, to be shortly succeeded by type 2, which then became the predominant type. The epidemic of 1916, however, has also been remarkable for an increase in the number of cases due to type 4."

Gordon summarises the meningococci from cases during 1916 as follows:

Type ...	1	2	3	4
Specimens	64	69	6	18

In comment on the above summary, I may point out that types 1 and 3 give a total of 70, and types 2 and 4 a total of 87. The former, 70 specimens, I regard as Group I, and the latter, 87 specimens, as Group II.

Apparently the meningococci responsible for sporadic cases and small outbreaks prior to the year 1914 were not so well defined in their serological characters as those obtained subsequently.

In reference to the epidemic of 1914-1915, the Medical Research Committee's Report says: "The reports from the Salisbury Plain area suggest not indeed that the Canadians imported a new disease into this country, for we have always had it with us in sporadic form, but that they did introduce a virulent strain of meningococcus and were in some degree responsible for its spread."

If the results of recent serological work in this country are compared with past records, the above suggestion finds some support. Arkwright (1909)² foreshadowed the division of meningococci into serological groups but his groups were not well defined nor limited to two. In his work on the epidemic of 1915 on the other hand, he found that only 5 out of 35 spinal strains failed to conform to one or other of two groups. Ellis during the same period was similarly successful in allotting his strains to two groups, and the work in this laboratory had a like result. Gordon³ in his previous work on the agglutination reactions of the meningococcus (1907) failed to find them of any value in the identification of the meningococcus. But recently he, like the above authors, readily identified two main groups into which 80 per cent. of his spinal strains fell.

¹ *Special Report Series*, No. 3, pp. 16-18.

² *Journ. of Hygiene*, ix.

³ *Report to the Local Government Board*, 1907.

It would be interesting to know if there had been a similar correlation between the types of meningococci in the naso-pharynx at these different periods.

There is unfortunately no information as regards the distribution of the meningococcus in the naso-pharynx of people in this country prior to the recent epidemic. Arkwright in his early investigation of contacts on Salisbury Plain did not find the meningococcus at all widespread: 4 positives out of 349 naso-pharyngeal examinations of contacts. The investigation of non-contacts conducted in the Board's Laboratory showed that during the month of April, 1915, meningococci were numerous in the throats of a section of the London population. Whether this result represented the normal seasonal incidence or was a special circumstance related to the epidemic prevalence cannot now be determined. As regards the serological characters of the pharyngeal meningococci of non-contacts, it has been found that the standard Group II sera agglutinate many more pharyngeal strains than the Group I sera and that the Group II antigens of the pharyngeal strains can be more readily identified with the similar antigens in the spinal strains than can the pharyngeal Group I antigens.

As an interesting parallel to the evolution of epidemic meningococci, the results of an investigation by Seligmann¹ on the bacteriology of dysentery in the German army may be quoted. He observed that at the height of the disease in 1915 and again in the following year the strains of dysentery isolated were almost exclusively typical, and it was only towards the decline of the epidemic in each season that the atypical forms made their appearance. Discovering at the end of the epidemic of 1915 that carriers of dysentery bacilli were rare, he determined, on the recurrence of the disease in 1916, to study the strains from the very earliest cases. His assumption was that if epidemic strains developed anew under the influence of climatic and other factors, the earliest strains would include amongst them undeveloped intermediate forms. He tested the suspicious strains from the first 34 cases as to their relationship to dysentery bacilli, and he found a considerable number of these diverged more or less from the recognised types; of the remainder, five were readily classed as typical Shiga and nine as Flexner bacilli. The results supported his original hypothesis, and in addition he was able to arrange the strains in such a way as to give a strong impression of stages in the evolution of the types of dysentery bacilli. The series was as follows:

- (1) A gas-forming bacillus related to *B. coli*.
- (2) Inagglutinable bacilli of the Flexner group without appreciable antigenic properties.

¹ *Centralbl. f. Bakteriol. Orig.* LXXIX. 71.

(3) Poorly agglutinable Flexner bacilli, possessing highly specific and individual antigens.

(4) Poorly agglutinable Flexner strains, the antigenic capacities of which closely resembled the normal Flexner type.

(5) Typical Flexner bacilli.

(6) Strains (*Doppelstämme*) which were agglutinated by both Flexner and Shiga sera and produced specialised sera showing relationship to (4).

(7) Poorly agglutinable Shiga-Kruse strains. .

(8) Typical Shiga-Kruse bacilli.

The author noted that there were many gaps, notably between Groups 1 and 2 and between Groups 6 and 7.

There are certain analogous features in the serological reactions of meningococci and dysentery bacilli. In both species there are two well-defined groups and a number of strains which exhibit characteristics of both groups. In the case of the meningococcus some of the latter strains have not only the agglutinability of both groups but also the agglutinogenic capacities. Dysentery strains corresponding to these would occupy the gap between (6) and (7), in Seligmann's series.

It seems to me difficult to conceive the typical Flexner antigen changing into the typical Shiga antigen. I should prefer to consider the intermediate strains as possessing simple forms of both Shiga and Flexner antigens, one or other of which by a process of chemical adaptation may develop greater complexity. Evolution thus takes place along two different lines, resulting in the formation of typical bacilli either of the Shiga or of the Flexner type.

Two alternative explanations of emergence of types in connection with the recent epidemic of cerebro-spinal fever may be discussed.

(1) Emergence occurred in one focus only, with subsequent spread and confusion with indigenous strains. The suggestion that a virulent strain was introduced into the country has been referred to. According to the scheme on p. 177 this meningococcus might be represented either as approximating to the hypothetically complete epidemic strain with both antigens in equal proportions and of equal complexity or as a strain less completely equipped but with the Group I component more developed than the Group II. On the latter assumption this strain, being alterable in type, might in its passage from naso-pharynx to naso-pharynx become resolved into two less complex strains, one with the Group I antigen in excess and the other with the Group II. The greater complexity of the former antigen would result in the greater range of variation which has been shown by strains of Group I. On the other hand the simpler form of Group II antigen would explain the occurrence of the large number of sub-groups in both the spinal and the naso-

pharyngeal series of apparently equal complexity. The frequent occurrence of the typical Group I strains in the spinal fluid compared with their comparative rarity in the naso-pharynx would be accounted for on the assumption that they were more adapted for invading the body in virtue of the possession of the more highly developed antigen.

This higher virulence however, is only relative, as the following considerations show. Three of the more complex Group I strains were found in the naso-pharynx of the 700 non-contacts examined. Since the incidence of cerebro-spinal fever in 1915 was .07 per 1000 of the general population of England and Wales and approximately half the cases would be due to Group I strains, it is obvious that the most virulent strains produce cerebro-spinal fever in a very small minority of the persons carrying such organisms in their throats.

The above hypothesis carries with it the assumption that carrier strains of meningococci spread throughout the country from a primary focus on Salisbury Plain, and that the different serological races are less complex variants of the original epidemic strain. One difficulty in supporting this hypothesis is that the existence of this complex epidemic strain, though suggested, has not actually been demonstrated.

(2) Emergence occurred by evolution in more than one focus. The Medical Research Committee's Report discusses the conception that in an epidemic of cerebro-spinal fever there may be a saprophytic spread of individual races which have attained greater virulence side by side with the domestic and relatively harmless strains indigenous to the locality. In this case evolution takes place along different lines, and one has to imagine some influence in action which sets in motion the process at a number of foci.

In the earliest serological investigations made during this epidemic Ellis¹ found both types represented in every epidemic focus he investigated and Arkwright¹ found no support for the view that the meningococci concerned in a particular outbreak were limited to one group. Such distribution may indicate that the two types were evolved simultaneously in the several foci; it is however equally consistent with a possible spread of both from a single focus.

The final decision between (1) and (2) rests probably with epidemiology, but the above evidence brought forward on the variations in the serological characters of the organisms concerned may perhaps be of some assistance.

Further light may be thrown on these questions in the future when an epidemic occurs after a period during which the disease has only

¹ *Brit. Med. Journ.*, 1915, II.

exhibited sporadic manifestations. In the meantime it will be interesting to study, as the present prevalence of cerebro-spinal fever gradually diminishes, the changes taking place in the serological characters of the strains of meningococci concerned. And, in the light of the evolutionary possibilities suggested by Seligmann in the case of dysentery bacilli and enunciated in more general terms by Andrewes and Horder, who consider that different species may still be in process of emergence from the same genus, one might speculate on the relationship to the meningococcus group of the naso-pharyngeal organism which cannot be accepted as a meningococcus but differs culturally from the true meningococcus only in the marked production of pigment. The few strains of this character which I examined exhibited no serological relationship to true meningococci. According to one's point of view, such strains might be considered as the possible starting point or the possible end point in the process of evolution of the meningococcus.

SUMMARY AND CONCLUSIONS.

The serological characters of 66 strains of meningococci obtained from the cerebro-spinal fluid in cases of meningitis have been studied. Serological tests of a similar nature have been applied to 86 strains of Gram-negative cocci, culturally identical with meningococci, obtained from the naso-pharynx of non-contacts. The two series have been compared in respect of agglutinability, agglutinogenic capacity, and absorptive capacity with the following results:

The majority of the spinal strains can be divided into two main groups by simple agglutination tests with selected spinal sera: about 27 can be assigned to Group I and 34 to Group II, the remaining five being relatively inagglutinable or equally agglutinated by sera of both groups.

If the naso-pharyngeal strains are grouped on the same principle, that is, according to the height of titre and consistency of reaction in the presence of the same standard group sera, two or three would be classed as Group I and about 50 as Group II; the remainder could not be classed on account of inagglutinability or equal agglutination with sera of both groups.

The naso-pharyngeal strains produce active agglutinating sera for spinal strains. Certain pharyngeal strains, resembling the atypical spinal strains in agglutinability and agglutinogenic capacity, produce sera which agglutinate without distinction spinal strains belonging to sepa

rate groups as shown by their agglutination with the standard spinal sera.

The naso-pharyngeal and cerebro-spinal strains which are agglutinated by Group II sera absorb the homologous agglutinin from those sera with equal readiness. On the other hand, the typical Group I sera are less readily and completely absorbed by the pharyngeal than by the spinal strains agglutinated by them. This difference has been found to be dependent upon variations in complexity of structure of the Group I antigen. Similar variations have been shown to occur in typical spinal meningococci during subcultivation.

Stated in general terms, the inter-relationship between spinal and pharyngeal strains is as follows:

The naso-pharyngeal strains are not serologically separate and distinct from the cerebro-spinal strains, and moreover have not been found to exhibit among themselves such serological differences as would justify the separation of any number of them into a class or classes, identical with each other and distinct from meningococci of cerebro-spinal origin. Consequently I can find no justification for the view that naso-pharyngeal meningococci of the non-contact differ as a class from strains which might be found within the immediate environment of cases of the disease. The occurrence of such organisms in considerable numbers among the non-contact population, though unexpected, is in accordance with epidemiological experience that the majority of cases of the disease cannot be traced to a previous focus.

The most pronounced of the serological differences which are found between spinal and pharyngeal strains are not greater in degree than the differences between individual spinal strains. Admittedly the meningococci of cerebro-spinal origin which exhibit so great divergence from the majority or the typical, are relatively few, but it is certain that the 66 specimens of cerebro-spinal origin investigated do not exhaust all the possibilities of variation. For this reason, as well as on account of the necessity of preparing an agglutinating serum from each atypical strain, it has not been possible to identify every naso-pharyngeal with a cerebro-spinal, that is, with a meningococcus of proved pathogenicity; but sufficient progress has been made to justify a general statement that the serological differences between the spinal and pharyngeal strains are not of a specific nature.

Although, as I have shown, the two series of meningococci belong to one and the same species, the comparative tests show that while a considerable number of the pharyngeal strains are serologically identical

with the majority of the spinal strains, there is a residue in each of the two series, much larger in the case of the pharyngeal, which is not capable of being identified in a complete serological sense with that majority. These residual pharyngeal strains are not identical with each other, but a few of them have been identified with the smaller number of the residual spinal strains.

It may be concluded from the above that a certain proportion of pharyngeal meningococci appear to effect a successful invasion of the meninges with relative infrequency, and there is a reasonable probability that the differential factor is one of virulence. There is thus suggested a correlation between virulence and serological reactions. The serological differences between strains of meningococci have been shown to depend upon variations in complexity of antigens as inferred from differences in range of absorptive capacity. The least complex strains are found more frequently in the naso-pharynx of the non-contact and in the meningitis of infants than in cerebro-spinal fever of adults. It may be concluded that virulence, in the sense of capacity to invade the meninges from the naso-pharynx, runs parallel with antigenic complexity. The most complex strains are the most virulent and occur most frequently at the height of an epidemic.

The less complex antigens do not bind the anti-bodies produced by the more complex. This fact is of obvious practical importance in the selection of strains for the production of therapeutic sera; each of the two group antigens should be represented in the most complex form available.

The naso-pharyngeal strains of Gram-negative cocci used in this investigation were selected solely on the grounds of identity in cultural and fermentation tests with meningococci of cerebro-spinal origin. Since the serological tests have confirmed the relationship of the former organisms to the latter, the conclusion is justified that meningococci form a well-defined species of Gram-negative cocci and can be identified in the naso-pharynx by careful application of cultural and fermentation tests.

ADDENDUM.

Since the preparation of the preceding part of the report the serological reactions of a further series of 24 spinal strains have been tested. For seven of the cultures I am indebted to Professor Andrewes. The remaining cultures were obtained from cerebro-spinal fluid of cases of meningitis sent to the Board's Laboratory for diagnosis from various

parts of the country during the period from February 28, 1917, to May 5, 1917. The same tests have been applied as were found useful in comparing the antigenic properties of different strains of meningococci in the main report. All the suspensions of the different strains were the same throughout. The results, which are summarised in the accompanying table, serve to illustrate several of the characteristic features of the inter-relationship between strains of meningococci demonstrated in the report. These will be referred to under the various headings.

A. Correlation between fermentation reactions and serological tests; the production of acid with maltose is associated with the presence of Group I antigen, and acid production with glucose with the presence of Group II antigen.

B. Division of spinal strains into two groups by the action of two agglutinating sera prepared with standard spinal strains, M 10 and M 18.

Demarcation of spinal strains into groups disappears when strains are tested with sera made from undifferentiated naso-pharyngeal strains, NP 44 and NP 43.

C. Group I strains exhibit gradation in antigenic complexity and corresponding variations in absorptive capacity for the agglutinins in Group I sera.

M 69 absorbs homologous agglutinin from three sera: M 10 serum (A agglutinin), M 17 serum (B agglutinin), and M 15 serum (C agglutinin).

M 67, 68, 70, 71 and 72 absorb A and C agglutinins.

M 76 and 79 absorb B and C agglutinins.

M 73, 74, 77, 78 and 75 absorb C agglutinin only, the first four partially and the last, M 75, completely.

M 80 absorbs B agglutinin partially.

D. Group II strains do not show the same gradation in absorptive capacity as the Group I strains. The majority absorb one or other of the four selected Group II sera, but several, M 86, 87, 88 and 81 fail to absorb any agglutinin completely from these spinal sera.

E. Spinal strains in both groups may absorb agglutinin from the naso-pharyngeal serum, NP 44.

F. The strains M 70, 69, 71, 79, 76, 75, 78, 90, 85 and 84 were obtained from a localised outbreak of cerebro-spinal fever at a camp¹. Seven belonged to Group I, showing every degree of absorptive capacity previously demonstrated; two absorbed agglutinin from the same Group II serum; one absorbed agglutinin partially from a Group I serum and completely from a Group II serum.

¹ In respect of the termination of the disease in these cases Dr Whitley kindly gave me the following information:

Of the 7 infected with Group I strains, 5 died and 1 recovered; in 1 the disease has become chronic and the result is yet in doubt.

Of the 3 infected with Group II strains 1 died and 2 recovered; strains with identical serological characters produced in one case a mild disease and in another a fulminating type.

SUMMARY OF FERMENTATION AND SEROLOGICAL REACTIONS OF 24 RECENT CEREBRO-SPINAL MENINGOCOCCI.

A	Simple agglutination Tests										E				
	B					C						D			
	Naso-pharyngeal					Absorption of Group I							Absorption of Group II		
	sera					sera									
Strain	Group I		Group II		serum	M 10 serum	M 17 serum	M 15 serum	M 18 serum	serum		M 32 serum	M 33 serum	Absorption of Naso-pharyngeal sera	Age of patient
	M 10	M 18	NP 44	NP 43						M 24	M 33				
M 67	M. > G.	1600	-	400	400	+	-	Partial	-
M 68	G. = M.	1600	-	400	800	+	-	+	-	NP 44 partial	Child
M 69	M. > G.	1600	-	400	200	+	+	+	-	18 years
M 70	M. > G.	1600	-	800	800	+	-	+	-	18 "
M 71	M. > G.	1600	-	100	-	+	-	+	33 "
M 72	G. = M.	800	-	400	200	+	-	+	-	NP 44 +	35 "
M 73	M. > G.	1600	400	100	-	-	-	Partial	-	...	-	-	-	...	17 "
M 74	...	800	-	800	-	-	-	Partial	-	13½ "
M 75	M. > G.	800	200	400	200	-	-	+	-	15 "
M 76	M. > G.	-	+	+	10 "
M 77	M. > G.	400	-	400	400	-	-	Partial	-	...	-	-	-	...	Adult
M 78	M. > G.	400	200	800	800	-	-	Partial	-	...	trace	trace	18 years
M 79	M. > G.	200	100	200	200	-	+	+	18 "
M 80	M. > G.	200	-	400	400	-	Partial	-	-	-	Adult
M 81	G. = M.	200	200	1600	800	-	...	-	Partial	...	Partial	-	...	NP 44 partial	...
M 82	G. > M.	-	1600	800	400	-	-	...	+	...	-
M 83	G. > M.	-	1600	800	800	-	-	-	+	...	-	-	...	NP 44 -	Infant
M 84	G. > M.	-	1600	1600	800	-	-	...	-	...	-	+	18 years
M 85	G. > M.	-	1600	200	100	-	...	-	-	...	trace	+	15½ "
M 86	G. > M.	-	800	800	400	-	-	-	-	...	trace	-	2½ "
M 87	M. = G.	-	800	400	200	-	-	Partial	Partial	...	-	-	23 "
M 88	G. > M.	-	400	400	400	-	...	-	-	...	Partial	-	5 "
M 89	M. = G.	-	400	800	800	-	-	...	-	...	Partial
M 90	M. > G.	-	100	400	400	-	-	Partial	+	...	NP 43 +	18 "

A FURTHER STUDY OF THE SEROLOGICAL REACTIONS OF MENINGOCOCCI FROM THE SPINAL FLUID AND THE NASO-PHARYNX, WITH SPECIAL REFERENCE TO THEIR CLASSIFICATION AND TO THE OCCURRENCE OF THE LATTER AMONG NORMAL PERSONS¹.

BY W. M. SCOTT, M.D.

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¹ Reprinted from *Reports to the Local Government Board on Public Health and Medical Subjects*, n.s., No. 114 (1917), by permission of His Majesty's Stationery Office.

INTRODUCTION.

In a previous report¹ I showed that micro-organisms microscopically and culturally indistinguishable from meningococci of pathogenic origin were present in the naso-pharynx of 30 out of 138 persons (22 per cent.) who had had no demonstrable connection with cases of cerebro-spinal fever. Many of these meningococcus-like organisms were also indistinguishable from known pathogenic strains by serological tests, *i.e.* they agglutinated like known pathogenic meningococci in high dilutions of anti-meningococcal sera; others, however, failed to react to a decisive extent, resembling in this certain pathogenic strains which also failed to give decisive results with any of the sera applied.

The serological tests recorded in that report showed, in addition, that great differences exist among cerebro-spinal as well as among naso-pharyngeal meningococci in their behaviour towards any one mono-valent agglutinating serum, but evidence was brought forward to show that the majority of the known pathogenic meningococci examined could be put serologically into two main groups, the members of each being closely alike and well distinguished from those of the other group. At the same time it was shown that there existed both cerebro-spinal and naso-pharyngeal strains which could not be satisfactorily identified by serological tests with either of these two main groups.

In continuing this investigation I have obtained additional strains for study, both from the spinal fluid of cases of cerebro-spinal fever and from the naso-pharynx of persons not associated with the disease, and I have endeavoured in particular to determine by further serological tests the biological relationships of the aberrant strains mentioned above to each other and to the apparently well-defined main groups. For this purpose, I have investigated their agglutinogenic and absorptive capacities, as well as their agglutinability: *i.e.* I have injected them into rabbits and tested upon both typical and atypical strains the agglutinating properties of the sera so produced, and I have compared aberrant with typical strains as regards their capacities of absorbing agglutinin from various sera.

The material employed for the purpose of this report consists of 60 strains of meningococci cultivated from spinal fluid, including 26 not dealt with in my previous investigation, and 71 strains obtained from the naso-pharynx, the majority from people not suspected of connection

¹ *Journ. of Hygiene*, xv. p. 464.

with cases of the specific disease, but others from direct contacts. Of these, 55 have been isolated since the completion of my former report. The work has been done in the Board's laboratory, and, as before, in consultation with Drs Eastwood and Griffith of the Board's pathological staff, to whom I wish again to express my indebtedness. I have also to thank various regimental medical officers for permission to take swabs from men under their care.

ORIGIN OF THE STRAINS INVESTIGATED.

Of the strains of cerebro-spinal origin 21 were isolated by Dr Arkwright, of the Lister Institute, during the epidemic of 1915; these were partially studied and described in my previous report (*loc. cit.*), and appear in the present description under the same designations A 1, A 2, etc. Twelve strains I owe to the kindness of Professor Andrewes and Dr Canti, of St Bartholomew's Hospital; these were isolated during the latter part of 1916, and with one exception were from infants under seven years. They are designated B 1, B 2, etc.; they are different from the strains from the same source described by Dr Griffith, except B 12, which is his M 77. Finally, 27 strains were isolated by myself, all but four being from specimens of cerebro-spinal fluid kindly sent me during 1915 and 1916 by Dr Foord Caiger, of the South-Western Isolation Hospital. These are designated C.S. 1, 2, etc., those described in the former report bearing the same numbers in this.

NASO-PHARYNGEAL STRAINS.

These are designated N 1, N 2, etc., the numerical order being determined by the alphabetical order of the names of the persons furnishing the strains and by the date on which each batch of swabs was taken. Strains N 1 to N 16 represent the survivors of those isolated in June and July, 1915, from out-patients attending Lambeth Infirmary, and have already been partially described in my former report. The following strains were collected whilst, in my capacity as local Medical Officer of Health, I was cooperating with the military authorities in the control of epidemic disease. Strains N 17 to N 22 were isolated on February 17, 1916, from soldiers in a garrison town in Kent; N 23 to N 27 were obtained on March 2, 1916, from soldiers in huts near a small village on the Kentish coast, while N 43 to N 49 were obtained from another section of the same battalion at this place on May 11, 1916. None of these had had any known connection with cases of cerebro-spinal fever, but

on May 24, 1916, two cases of the disease occurred in this battalion, and strains N 54 to N 71, isolated on May 26, 1916, were from contacts occupying the same huts as the two cases. Strains N 28 to N 30 were isolated on March 23, 1916, from the personnel of a medical inspection room attached to a battalion in camp in a rural parish in Kent, while strains N 31 to N 42 were taken from soldiers of this battalion on March 28, 1916. None of these had had any direct connection with cases of cerebro-spinal fever, but a soldier from the battalion developed the disease on March 20 while absent on leave, and there was also a civilian case, a child, on the same date, in a house in which two men from the same body were billeted. No other cases were reported to me among or in connection with these soldiers afterwards.

Finally strains N 50 to N 52 were obtained from one civilian and two military contacts of two cases which occurred in billets on March 20 and April 5, 1916, in the town of S—, Kent, while strain N 53 was isolated from a soldier, a positive contact who had been pronounced a chronic carrier by the military authorities concerned.

Among my 71 naso-pharyngeal cases the first 49 strains, N 1 to N 49, are thus derived from non-contacts, while 22, N 50 to N 71, are from contacts; 17 are from civilians, nearly all of adult age, and 54 from soldiers; 16 were isolated during June and July, 1915, and the rest during the first half of 1916.

MORPHOLOGICAL AND CULTURAL CHARACTERS.

In this connection I have no reason to supplement or modify the details contained in my former report regarding the appearance of colonies on the primary plates; as before, a large proportion of the naso-pharyngeal strains fermented glucose more strongly than maltose; with some, indeed, the fermentation of maltose was extremely feeble on first isolation.

AGGLUTINATION TESTS.

Sera employed.

As before, monovalent sera alone have been employed, all from rabbits. Nineteen strains altogether have been used for their preparation, and the sera produced are designated by the same symbols as the strain injected. Two were prepared by injection of presumed pathogenic meningococci from the naso-pharynx of acute cases of cerebro-spinal fever. Of these P 1 is the strain which produced the serum "Boscombe" referred to in my former report, while P 2 is "Clayton." Nine were

prepared with strains of cerebro-spinal origin, and are known by the corresponding titles, C.S. 8, C.S. 14, C.S. 16, C.S. 20, A 2, A 10, A 13, A 17, A 24. Of these strains C.S. 8 and A 2 were used in producing the sera of the former report named "Smith" and "Chandler."

Eight naso-pharyngeal strains from non-contacts were employed, and the respective sera are entitled N 1, N 2, N 7, N 10, N 13, N 19, N 29, N 48.

With all but one of these 19 strains I obtained sera agglutinating the homologous coccus completely in dilution of 1-1000 or over; in the case of strain N 7, in spite of prolonged immunisation, the titre failed to surpass 1-800. In this strain abnormal toxicity of the culture caused the death of several rabbits during immunisation, so that a satisfactory serum was not produced; this was the case also with several other strains, both naso-pharyngeal and spinal, the sera obtained being of too low titre for use.

Technique of preparation of sera.

Antigens. The bacterial growth was obtained from slopes of Kutscher's medium or glucose-ascitic agar of 24 hours' incubation, and was injected intravenously while still alive and fresh. The dose employed was increased during immunisation from half a slope to two slopes. In the case of the more "toxic" strains on first injection even a loopful of growth, say 10 mg., was fatal within 48 hours. In some cases the bacterial growth from 24 hour egg slopes was used, but the progress of immunisation was unsatisfactory and eventually in all cases Kutscher or glucose-ascitic agar was employed. Some of the sera did not reach the final high titre until as many as 20 doses had been given, spread over six months. I found that a period of rest in the process of immunisation was useful, since the titre which had before been stationary began to rise satisfactorily again on resumption.

The object of this prolonged immunisation was to procure sera of high titre so that a considerable range of dilution might be employed for comparative tests on different strains, and also so that possible "group agglutinins" might be reduced as much as possible in amount relative to the agglutinins more special for the strain.

Technique of agglutination tests.

The macroscopic method was again used throughout, the mixtures of serum and coccal suspension being incubated for 24 hours at 55° C.

Suspensions were made from glucose-ascitic agar cultures of 24 hours' growth sown from 24 hour egg cultures, the growth being weighed moist and suspended in phenol-saline (0.5 per cent. phenol) at the rate of 2 mg. per c.c.; these were then heated for an hour at 65° C. and kept as stock suspensions. They remained agglutinable for many months, the agglutinability in general tending to increase rather than diminish. In addition, freshly-prepared suspensions (unheated) of the same strength were tested from time to time. These suspensions tended fairly rapidly to become inagglutinable, the rate of change varying very much with different strains.

The mixtures were put up in Durham's tubes calibrated in two equal portions, each containing about 0.3 c.c.; the diluted serum was first put in, then the suspension run in with violence so as to ensure mixture. The use of these small tubes effects a considerable economy in the amount of serum necessary for tests, while providing a sufficient column of suspension for estimating changes resulting from agglutination.

Agglutination of meningococci of cerebro-spinal origin.

In Table I, subjoined, are given the highest titres at which complete agglutination resulted in the case of 60 meningococci of spinal origin with monovalent sera produced by nine strains isolated from adult cases of the specific disease during the epidemic season, two being from the naso-pharynx, and seven from the spinal fluid. As will be seen later, the titre varies with different suspensions of the same strain, but the figures here represent the maximum agglutinability of the various strains with each serum, "maximum" because the most sensitive suspensions were used (old stock heated suspensions) and because the highest value attained in any one experiment was selected.

This table demonstrates the relationships to each other of strains of spinal origin as shown by agglutination. In my former report I showed that agglutination tests with two monovalent sera, "Boscombe" and "Clayton," divided the spinal strains sharply into two groups, the first group agglutinating completely at 1-100 to 1-800 with "Boscombe" serum and either not at all or very slightly at 1-100 with "Clayton" serum, while with the second group the behaviour was reversed. With the stronger sera here employed this grouping is still more evident. Taking the first and last columns, headed sera P 1 and P 2 ("Boscombe" and "Clayton"), it will be seen that the first 24 strains agglutinate to the full titre or nearly with the former and, with two exceptions of 1-500, agglutinate only up to 1-100 or less with the latter; on the other hand

TABLE I. *Agglutination of spinal strains of meningococci with sera produced by known pathogenic strains.*

Serial No.	Strain	P 1 Serum Titre 1-2000	A 17 Serum Titre 1-1800	C.S. 8 Serum Titre 1-2000	A 13 Serum Titre 1-1500	A 10 Serum Titre 1-1500	A 24 Serum Titre 1-1000	C.S. 14 Serum Titre 1-1500	C.S. 16 Serum Titre 1-1500	P 2 Serum Titre 1-2000
1	C.S. 1	2000	1500	1000	100	100	100	100	100	100
2	C.S. 2	2000	2000	1500	100	100	o	100	100	100
3	C.S. 3	2000	1500	1000	100	500	100	o	100	100
4	C.S. 5	2000	2000	1000	100	500	100	o	100	500
5	C.S. 6	2000	1500	1000	100	1000	100	o	100	100
6	C.S. 7	2000	1000	500	o	500	100	o	100	100
7	C.S. 17 (1)	2000	2000	500	500	500	o	o	o	100
8	C.S. 18	2000	2000	1500	100	100	100	o	o	100
9	C.S. 21	1500	1500	1000	100	100	100	o	100	100
10	C.S. 23	2000	1000	1000	100	100	o	o	o	500
11	C.S. 24	2000	1000	1000	100	100	o	o	o	500
12	C.S. 25	2000	1500	1000	500	100	50	o	o	100
13	C.S. 27	2000	2000	1500	500	500	100	o	o	100
14	A 3	2000	1500	500	100	500	100	o	100	100
15	A 4	2000	2000	1500	100	100	100	o	100	500
16	A 6	2000	1500	1000	500	500	50	o	o	100
17	A 9	2000	2000	1000	100	500	100	o	100	100
18	A 11	2000	2000	1000	100	500	100	o	100	100
19	A 12	2000	2000	1000	100	100	100	o	100	100
20	A 14	2000	2000	1000	500	500	100	o	100	100
21	A 15	2000	2000	1000	100	100	100	o	100	100
22	B 2	1500	2000	1000	100	100	o	o	o	o
23	A 18	2000	1500	1000	100	500	100	o	100	100
24	A 17	1500	1800	1000	100	100	o	o	o	100
25	C.S. 8	500	500	2000	100	100	100	o	o	o
26	A 7	500	500	2000	100	100	100	o	o	o
27	C.S. 20	500	1000	2000	100	500	100	100	o	100
28	C.S. 4	500	1500	1500	1500	1000	100	100	100	100
29	A 13	500	500	1000	1500	1000	100	100	100	100
30	A 23	500	100	o	1000	1500	100	100	500	1000
31	A 1	500	1500	1000	500	500	100	100	500	100
32	A 16	100	500	1000	100	500	100	o	o	o
33	A 2	100	500	500	o	500	500	100	500	100
34	B 12	100	500	500	100	500	o	o	o	o
35	A 10	500	1000	1000	500	1500	50	100	500	100
36	A 24	o	o	100	100	500	1000	1000	1500	2000
37	A 22	o	o	o	o	100	1500	1000	1500	1500
38	C.S. 9	o	100	o	o	o	500	1000	1500	2000
39	C.S. 10	o	100	o	o	o	100	1000	1500	1500
40	C.S. 11	o	100	o	o	100	500	1000	1500	2000
41	C.S. 12	o	o	o	o	100	100	1000	1200	2000
42	C.S. 14	o	100	o	o	500	100	1500	1500	1500
43	C.S. 15	o	100	o	o	100	100	1000	1500	1500
44	C.S. 16	o	o	o	100	500	100	1000	1500	1500
45	C.S. 17 (2)	100	o	o	100	o	100	1000	1200	1500
46	C.S. 19	o	o	o	o	o	100	500	500	2000
47	C.S. 22	o	100	o	o	o	500	1000	1500	2000
48	C.S. 26	o	o	100	o	o	-	1000	1500	2000
49	A 20	o	o	o	o	o	100	1000	1500	1500
50	B 7	o	o	o	o	100	100	1500	1500	1500
51	B 6	o	o	o	o	100	100	1000	1200	1500
52	B 9	o	o	o	o	o	o	1000	1000	1500
53	B 11	o	100	o	o	o	50	500	1000	1500
54	B 4	o	o	o	o	100	100	1000	800	1000
55	A 25	o	100	o	o	100	100	1000	500	1500
56	B 8	o	100	o	o	100	50	500	100	500
57	B 1	o	o	o	o	100	o	500	100	500
58	B 3	o	100	o	o	100	o	100	100	500
59	B 5	o	o	o	o	100	o	100	o	100
60	B 10	o	100	o	o	100	100	o	100	o

20 other strains, 36 to 55, agglutinate up to the full titre or nearly with serum P 2 and slightly or not at all with serum P 1. Thus in the case of 44 strains a sharp division into groups exists on the strength of agglutination.

This leaves 16 in which agglutination by these group sera left the classification in doubt. Eleven of these, numbers 25-35, show agglutination with the main Group I serum varying from 100 to 500 and with the main Group II serum from 0 to 100, with one exception which agglutinates to 1000 with the latter serum.

The last-mentioned strain might be classed as Group II, but in the case of all the others the agglutination results leave one either entirely in doubt or with a slight leaning to place them in Group I. Finally five strains, numbers 56-60, do not agglutinate at all with Group I serum and with Group II serum agglutinate at most up to 1-500. The tendency would be to place these in Group II, but again the position must remain in doubt since agglutination to 1-500 with such strong sera cannot be regarded as of decisive significance.

On looking now at the columns under A 17 serum and C.S. 16 serum exactly the same results are obtained except that with A 17 there are greater variations in the high titres obtained with the strains already placed in the main Group I, *e.g.* three strains reach only 1-1000, while nine reach 1-1500, and twelve attain the full titre of 1-2000; further, some of the strains which leaned towards Group I now show more definite alliance, reaching dilutions of 1-1000 in two cases and 1-1500 in other two; the strains 56-60 which inclined to Group II are now almost entirely indifferent, reaching titres of 1-100 at most, a titre which is also attained by the Group I strains with the Group II serum.

Sera A 17 and C.S. 16 thus do not alter the subdivision already noted, and the strains producing them must be regarded as examples of Group I and Group II respectively.

Serum C.S. 14 is closely comparable with C.S. 16 in its effects and picks out again the Group II strains, agglutinating them to approximately full titre.

The results of agglutination with the other sera, C.S. 8, A 13, A 10 and A 24, may be summed up as follows: the first three, prepared with strains already placed as inclining towards Group I, agglutinate Group I strains better than Group II and pick out in each case a small number of the former strains by agglutinating them to the full titre of the serum, while serum A 24, prepared with a strain apparently typical of Group II, agglutinates both groups indifferently, but neither well;

it picks out, however, one other Group II strain besides itself as reacting to the full titre. These two strains evidently form a sub-group of Group II although their agglutination reactions with the other Group II sera give no evidence of their highly individual character as shown by their behaviour towards serum A 24.

The agglutination tests leave unidentified eight strains which react feebly with all the sera, but one of these, strain A 2 (number 33) has been used to produce a serum, the action of which will be described in a later part of this report. In general it agglutinates Group I strains better than Group II, thus confirming its position as near Group I, but it agglutinates none of the spinal strains, except itself, to high titre, and may therefore represent an aberrant strain or may belong to a natural group of which chance has determined that no other representative should fall into my hands.

If one makes a general survey of Table I it may be noted that, if it be read from the top left-hand corner to the bottom right-hand corner, lines drawn from the top of column C.S. 8 to line 36 in column P 2 and from line 25 in column P 1 to the foot of column C.S. 14 include practically all the high agglutination titres between them.

The explanation of this is the fact that the middle of the horizontal line on which the sera are placed is occupied by sera surmised to represent strains intermediate between Group I and Group II, whilst the sera typical of these two groups occupy respectively the left- and right-hand ends; the middle of the vertical line on which are distributed the various strains is occupied by strains similarly surmised to be intermediate. The striking arrangement of the high titres which results in Table I testifies to the correctness of the surmise, and indicates that the agglutinogenic action of these intermediate strains is in agreement with their reactions to the main group sera.

In the case of eight strains out of the 60, classification with the sera employed has failed owing to their poor response to all. Three of these strains appear to incline more to Group I than to Group II, while with the remaining five the inclination is reversed. Each of these sets may represent a true sub-group, or they may consist of highly specialised individuals.

This question could only be answered by preparing sera with each, and testing the agglutinating properties of these sera against a representative selection of strains, *i.e.* by determining their general and special agglutinogenic action.

Variations in agglutinability of the meningococcus in culture.

Special attention to this point was evidently necessary, since neglect in taking account of it might lead to serious error not only in the more purely scientific question of making a classification but in the very practical one of estimating the value of particular anti-meningococcus sera as remedial agents in infections with particular strains of the meningococcus.

As has been stated, heated suspensions of the various strains remain very stable in their agglutinability, the only change, if any, being a gradual one towards increase of sensitiveness, not only towards the sera agglutinating originally to a high titre but also to those agglutinating originally only to a slight extent. In other words, heating the suspension fixes permanently the agglutinating properties which the cocci happen to possess in the fresh unheated state either as a result of the strain's true position among meningococci or as the result of a temporary variation. But such temporary variations between different cultures of the same strain occur to such an extent that suspensions made at different time from subcultures on Kutscher's medium or ascitic agar of strains preserved on egg at 37° C. show very wide divergence.

A good deal of time was spent on trying to correlate these divergences with the differences seen in colonies of the same strain on the same plate. These differences are particularly striking when material taken from an old egg culture (three weeks old or more) is plated out on serum or ascites media. On such plates colonies of two types appear in varying proportion; one type is translucent or very finely granular, resembling in appearance and structure the typical colony as isolated directly from the naso-pharynx or the spinal fluid; the other is opaque, coarsely granular and easily distinguishable. Some colonies are apparently of mixed type and show sharply defined sectors of translucent growth in the opaque disc. After one or two subcultures on serum media these coarsely granular colonies yield only the translucent type of growth, but cultures on egg from single colonies, whether translucent or granular, show after three weeks or so the same mixture on replating.

On investigating the serological reactions of these different types of growth it was found that in some cases the opaque type consisted of relatively inagglutinable cocci, while the translucent type gave the reactions regarded as normal for the strain; but in other cases the behaviour was reversed, and the conclusion I came to was that no correla-

tion between type of growth and agglutinability existed. The same holds true for differences in the size of colonies on the same plate.

The cause for the appearance of the two types of colony may be that in an old egg culture certain cocci have become so altered in the conditions of their growth by existence on an exhausted medium that when given an opportunity of forming colonies on fresh material they are unable at first to grow in the manner typical of meningococci from young and vigorous cultures.

In any case, their rapid reversion to type in this matter indicates that no profound mutation has occurred.

But these observations are of significance in showing that the varying agglutinability of different suspensions of the same strain may be easily explained by the predominance in them either of highly agglutinable or of feebly agglutinable cocci, according as the culture used for the suspension starts from cocci producing readily agglutinable colonies or from those producing the reverse.

In Table II examples are given of the different titres shown by the same strain with different fresh suspensions; all these suspensions were of the standard strength, 2 mg. per c.c. In the case of columns (3) and (4) they were made by subculturing on ascitic-agar single colonies of the different appearances described; column (3) shows the results obtained with the translucent variety and column (4) those with the opaque. Columns (1) and (2) represent growth from colonies of typical appearance from young cultures. The Arabic numerals indicate the dilution of serum at which complete agglutination occurred, while the symbols +, ++, and +++ indicate degrees of agglutination short of complete at 1-100.

It will be observed that at different times with both groups of strains the titre may vary from 1-100 to 1-1,500, *e.g.* with strains A 3 and C.S. 12, and that although in general the variation does not reverse the placing of the various strains in the two main groups, yet in many instances, *e.g.* A 3, A 4, A 6, A 17, C.S. 4, etc., the difference in titre shown, when minimum and maximum agglutinations with the respective group sera are compared, is not such as to give a definite answer as to serological grouping. Hence chance may readily determine that a given strain, though definitely of the main Group I as seen by more extensive tests, may appear as the result of a single test either quite doubtful or even tending towards the wrong group.

The variation just illustrated may, and probably does, depend on differences in the different suspensions of an extrinsic character, *i.e.* in

TABLE II.

Variations in agglutination with fresh suspensions.

Strain	Serum P 1				Serum P 2			
	1	2	3	4	1	2	3	4
C.S. 1 . .	1500	500	1500	—	++	+++	100	—
C.S. 2 . .	1500	1000	1500	1500	+++	100	100	100
C.S. 3 . .	1500	1500	1500	—	100	100	100	—
C.S. 5 . .	1000	1500	1500	—	++	100	500	—
C.S. 6 . .	1000	500	500	1500	o	+++	++	100
C.S. 7 . .	1500	500	500	1000	+++	+++	+++	100
C.S. 17 (1) . .	1500	1000	500	1500	100	+++	++	100
C.S. 18 . .	500	1000	1500	—	+	+++	100	—
C.S. 23 . .	1500	500	1500	1500	+	++	100	100
C.S. 24 . .	500	1500	1500	—	++	+++	100	—
A 3 . .	1500	100	1500	1500	+++	+++	++	+++
A 4 . .	1500	1000	500	—	500	++	++	—
A 6 . .	500	500	100	500	+++	+++	+++	+++
A 9 . .	1500	1500	1500	—	+++	+++	++	—
A 11 . .	1500	100	1500	—	+++	+++	++	—
A 12 . .	1500	500	—	—	100	+	—	—
A 14 . .	1000	1500	—	—	+++	100	—	—
A 15 . .	1000	1500	1000	—	++	100	++	—
A 18 . .	1500	1500	1500	—	100	+++	++	—
A 17 . .	+++	500	—	—	+	+++	—	—
C.S. 8 . .	100	100	o	100	+	+	++	o
A 7 . .	1000	100	—	—	+++	+	—	—
C.S. 20 . .	500	100	500	—	++	++	+++	—
C.S. 4 . .	o	100	100	100	100	o	+++	100
A 13 . .	100	100	100	100	100	++	+++	100
A 23 . .	o	o	—	—	500	++	—	—
A 1 . .	1000	100	o	—	100	100	o	—
A 16 . .	100	100	+	—	+	++	++	—
A 2 . .	++	100	++++	++	100	100	100	100
A 10 . .	100	100	—	—	100	100	—	—
A 24 . .	o	o	o	o	1500	100	100	500
A 22 . .	o	o	—	—	1500	1000	—	—
C.S. 9 . .	o	o	o	+	1000	100	1500	1500
C.S. 10 . .	o	o	o	—	1000	100	1000	—
C.S. 11 . .	o	o	o	—	1000	500	1500	—
C.S. 12 . .	o	o	o	+	1500	100	1500	500
C.S. 14 . .	o	o	o	o	1000	100	1000	1000
C.S. 16 . .	o	o	o	—	1000	1000	1000	—
C.S. 19 . .	o	o	o	—	1000	1500	1500	—
C.S. 22 . .	o	o	o	o	1500	1000	100	1500
A 20 . .	o	o	o	o	1500	1000	500	500
A 25 . .	o	++	o	o	1000	100	1000	1500

the physical condition of the cocci composing them, and not on intrinsic differences of chemical composition such as differentiate the groups. But one strain in my possession, strain C.S. 17, has shown differences of the latter type. Originally on isolation it agglutinated to the full titre with the main Group I serum, and only faintly with that of Group II; when a fresh emulsion was tested a month later, the behaviour was exactly reversed; two months later it again became a typical Group I strain, giving only traces of agglutination with Group II serum. As a result, I have now in my possession two strains isolated from the same colony on a plate inoculated with cerebro-spinal fluid; one strain C.S. 17 (1) behaves in all respects like the 24 strains making up the main Group I, while the other, C.S. 17 (2), is almost equally characteristic of Group II.

The significance of this apparently profound change will be discussed later with special reference to the possibility of original mixture of the two strains.

It may safely be assumed, I think, that if differences of the degrees described occur in strains during culture on artificial media, they are still more likely to occur under the changing conditions of the susceptible or resistant human body.

Further examples of these serological changes will be given when absorption tests have been discussed, since, in estimating the degree of importance to be attached to alteration in serological reactions, agglutination results are not sufficiently decisive; the absorption of agglutinin from a particular serum, it is generally held, is the most satisfactory criterion for establishing identity of an unknown strain with the strain producing the serum.

Classification by agglutination.

I have tried to show in Table I that by agglutination reactions alone it is possible to divide this collection of 60 spinal strains into two main groups supplemented by at least five sub-groups more or less nearly allied to Group I, one sub-group actually within Group II and one probably related to it. The term sub-group in each case indicates merely that the strains representing it are numerically less important than those of the main groups; it does not indicate that the sub-groups lie within the main Groups I and II; they may possess equally pronounced individuality, and all that can be said is that sub-groups 1 to 5 are more nearly allied to Group I while sub-group 7 inclines to Group II. In Table III the reasons are set out for separating each of these sub-groups, as also the numbers of the strains which go to make them up.

TABLE III.

Analysis of Table I showing expanded classification of spinal strains in relation to the two main groups.

No. of Group or Sub-group	Strains composing these: Nos.	Relationships to the main groups		Characteristics peculiar to each set of strains classed as identical
		Group I	Group II	
Main Group I	1-24	These strains constitute this group	Slight and indecisive agglutination with Group II sera	Agglutination to approximately full titre with Group I sera
Sub-group (1)	25, 26, 27 (C.S. 8, A 7, C.S. 20)	Agglutinated to 1-500 with main Group I sera	Agglutinated to 1-100 only with Group II sera	Agglutination to full titre with serum C.S. 8, prepared with strain No. 25
Sub-group (2)	28, 29 (A 13, C.S. 4)	Agglutinated to 1-500 with main Group I sera	Agglutinated to 1-100 only with Group II sera	Agglutination to full titre with serum A 13, prepared with strain No. 29
Sub-group (3)	30, 35 (A 23, A 10)	Agglutinated to 1-500 with main Group I sera	Agglutinated to 1-500 with Group II sera	Agglutination to full titre with serum A 10, prepared with strain No. 35
Sub-group (4)	33 (A 2)	Agglutinated to 1-500 with main Group I sera	Agglutinated to 1-500 with Group II sera	Agglutination to full titre with serum A 2, prepared with strain No. 33; no other spinal strain so agglutinated
Sub-group (5)	31, 32, 34 (A 1, A 16, B 12)	Agglutinated to 1-500 or higher but not to full titre with main Group I sera	Agglutinated below 1-500 with Group II sera	Fail to agglutinate to full titre with any spinal sera: ? identical with each other: ? separate individual groups
Main Group II	36 to 55	Agglutinated to slight and indecisive extent by main Group I sera	These strains constitute this group	Agglutination to approximately full titre with Group II sera
Sub-group (6) within Main Group II	36, 37 (A 24, A 22)	As above	Apparently indistinguishable	Agglutination to full titre with serum A 24 prepared with strain No. 36
Sub-group (7)	56-60 (B 8, B 1, B 3, B 6, B 10)	As above	Agglutinated to 1-500 or less with Group II sera	Fail to agglutinate to full titre with any spinal sera; agglutinate better with Group II than with Group I: ? identical with each other: ? separate individual groups

But in practice such classification by agglutination alone is out of the question. It would involve repeated testing of each strain to be classified; the tests would have to be performed with several sera, each known to be different, and strains of established classification would have to be tested at the same time for comparison and as controls.

A single test with selected sera would be insufficient because the agglutination titre of a particular strain for a particular serum may vary within wide limits with suspensions prepared at different times: one suspension may reach even less than half the titre attained by another, and a single indecisive result with a particular serum cannot be regarded as excluding membership of the corresponding group. Furthermore, with certain sub-group sera, *e.g.* serum C.S. 8, the agglutination reactions of some strains foreign to the sub-group differ on the average only slightly from those actually belonging to it, the differences being well within the limit of possible variations.

It will be seen, however, in the following section that by the test for absorption of agglutinin the classification suggested by the agglutination reactions of Table I and defined in Table III receives a considerable amount of support, the differences brought out by absorption being much sharper and less subject to variation.

ABSORPTION TESTS.

Technique. My general plan of procedure has been to suspend a weighed quantity of the bacterial growth (from cultures on ascitic agar of 24 hours at 37° C.) in a given amount of serum diluted 1-50. The quantity chosen was that which, with the strain homologous to the particular serum, was necessary to reduce the agglutinating power for itself to nearly *nil*—*i.e.* till the serum, which before absorption agglutinated its homologue, say, to 1-1500, failed to give complete agglutination at 1-100 (though preferably giving distinct traces of agglutination at this dilution). This quantity varied with different sera from 5 mg. to 20 mg. of culture per c.c. of serum diluted 1-50. The mixture was kept in the ice-chest overnight or longer as convenient, a control specimen of the same serum dilution being similarly treated. The cocci were removed by prolonged centrifuging, and the clear fluid was then tested for persisting agglutinin on a suspension of the strain producing the serum; usually other strains more or less closely allied to this were used as test suspensions at the same time.

In a few cases stored suspensions of cocci were employed for absorption instead of the fresh growth, but they were rather unsatisfactory owing to the difficulty of removing them with the centrifuge in cases where agglutination in the mixture was incomplete. The fresh suspensions, on the other hand, even when not agglutinated, were readily removed by the high-speed centrifuge, giving clear supernatant fluid with which to test agglutination.

Agglutination was tested with this fluid undiluted and with increasing dilutions of it up to the maximum known to give complete agglutination in the case of the control specimen, so that degrees of absorption were detectable from "complete," where only traces of agglutinin remained, to "nil," where the serum before and after absorption gave in each case complete agglutination at the highest dilution.

GROUPS AND SUB-GROUPS.

The main Group I.

In Table IV, subjoined, are given the degrees of absorption from the main Group I serum, P 1, with various spinal strains representing this main group (*vide* Table I), and also with strains more or less closely allied to this group (*vide* Table III); as controls two strains were taken, which on agglutination belonged to Group II. The quantity of culture employed for absorption was in each case 8 mg. suspended in 1 c.c. of P 1 serum diluted 1-50. The suspensions used for testing the persistence or removal of agglutinin were old stock suspensions of high agglutinability.

The symbol C, when used to indicate agglutination, means that complete deposit of the suspended cocci was found, the supernatant fluid being free from turbidity; the symbols + + +, + +, and + indicate diminishing amounts of deposit, the supernatant fluid remaining more or less turbid.

In the columns headed absorption the symbol C indicates that absorption was regarded as *complete*, complete absorption being taken to have occurred when even the 1-100 dilution failed to agglutinate the test suspension completely; absorption designated as + + + means that, though agglutination was still complete at 1-100, it was definitely incomplete at 1-600; while + indicates that agglutination, though complete at 1-600, was incomplete at 1-1000.

It will be seen that the first 24 strains, P 1 to B 2, which (*vide* Tables I and III) were put in the main Group I in virtue of their high agglutination with serum P 1, give complete, or almost complete, absorption of the agglutinin acting on strain P 1, while those strains which showed relationship but not identity with Group I—and also the control Group II strains—remove either small amounts or none. Similarly, the agglutinin present in P 1 serum for another member of the main Group I is in general removed completely by the main Group I strains and barely affected by the others.

TABLE IV.

Absorption of agglutinin from serum P 1.

Serum	Agglutination with Homologous Strain P 1					Agglutination with Related Strain C.S. 3					Absorption of Agglutinin for Homologous Strain P 1	Absorption of Agglutinin for Strain C.S. 3
	100 C	600 C	1000 C	1400 C	2000 +++	100 C	600 C	1000 C	1400 C	2000 +++		
Control unabsorbed												
Absorbed by Strain												
P 1 . . .	+	o	o	o	o	+	o	o	o	o	C	C
C.S. 1 . .	+	o	o	o	o	+	o	o	o	o	C	C
C.S. 2 . .	+	o	o	o	o	+	o	o	o	o	C	C
C.S. 3 . .	+++	trace	o	o	o	+	o	o	o	o	C	C
C.S. 5 . .	C	++	o	o	o	++	o	o	o	o	+++	C
C.S. 6 . .	C	++	o	o	o	++	o	o	o	o	+++	C
C.S. 7 . .	++	o	o	o	o	+	o	o	o	o	C	C
C.S. 17 (1) .	C	++	o	o	o	+	o	o	o	o	+++	C
C.S. 18 . .	++	o	o	o	o	++	o	o	o	o	C	C
C.S. 21 . .	C	++	o	o	o	++	o	o	o	o	+++	C
C.S. 24 . .	+	o	o	o	o	o	o	o	o	o	C	C
C.S. 25 . .	+	o	o	o	o	o	o	o	o	o	C	C
C.S. 27 . .	+	o	o	o	o	o	o	o	o	o	C	C
A 3 . . .	C	o	o	o	o	o	o	o	o	o	+++	C
A 4 . . .	++	o	o	o	o	o	o	o	o	o	C	C
A 6 . . .	C	o	o	o	o	++	o	o	o	o	+++	C
A 9 . . .	+++	o	o	o	o	+	o	o	o	o	C	C
A 11 . . .	+	o	o	o	o	o	o	o	o	o	C	C
A 12 . . .	+	o	o	o	o	o	o	o	o	o	C	C
A 14 . . .	C	o	o	o	o	++	o	o	o	o	+++	C
A 15 . . .	C	++	o	o	o	C	+	o	o	o	+++	+++
A 18 . . .	C	o	o	o	o	o	o	o	o	o	+++	C
A 17 . . .	C	+	o	o	o	C	++	o	o	o	+++	+++
B 2 . . .	C	o	o	o	o	++	o	o	o	o	+++	C
C.S. 8 . .	C	C	+++	++	+	C	C	C	C	++	+	trace?
A 7 . . .	C	C	C	C	++	C	C	C	C	++	trace?	trace?
C.S. 20 . .	C	C	C	C	++	C	C	C	C	++	trace?	trace?
C.S. 4 . .	C	C	+++	++	+	C	C	C	C	++	+	trace?
A 13 . . .	C	C	+++	++	+	C	C	C	C	+	+	trace?
A 1 . . .	C	C	+++	+	o	C	C	C	C	+	+	trace?
A 2 . . .	C	C	C	++	o	C	C	C	C	+	trace	trace?
A 16 . . .	C	C	++	+	o	C	C	C	+++	++	+	trace
B 12 . . .	C	C	C	C	+	C	C	C	C	+	trace?	trace?
B 3 . . .	C	C	C	C	+	C	C	C	C	+	trace?	trace?
A 10 . . .	C	C	C	C	+++	C	C	C	C	++	o	trace?
C.S. 14 . .	C	o	C	C	+++	C	C	C	C	++	o	trace?
*N 19 . .	++	o	o	o	o	+	o	o	o	o	C	C

* To avoid repetition of the table the absorptive capacity of N 19 is inserted here and will be discussed on p. 231 in its place as a naso-pharyngeal strain.

This result, which has been many times repeated, is very clear and definite; the minor variations in absorptive capacity, indicated in this table among the different strains within the main group, have not been reproduced with regularity in duplicate experiments, and probably indicate only slight temporary alterations in individual culture masses of the various strains. This main Group I is thus well defined and relatively homogeneous; and I may note incidentally that similar behaviour in the case of certain coli-form bacilli of the "food-poisoning" group has been used by some authorities as the criterion for establishing biological species.

In the following table (Table V) are indicated the results of absorption from another main Group I serum, the serum produced by strain A 17, which, though irregular in its agglutination with serum P 1 (*vide* Table II), yet at times reaches the full titre, and, as has just been seen, gives almost complete absorption of agglutinin from it.

TABLE V.

Absorption of agglutinin from serum A 17.

Serum	Agglutination with Homologous Strain A 17				Agglutination with Related Strain C.S. 2				Absorption of Agglutinin for Homologous Strain A 17	Absorption of Agglutinin for Strain C.S. 2
	100 C	500 C	1000 C	1500 C	100 C	500 C	1000 C	1500 C		
Control unabsorbed										
Absorbed by Strain										
C.S. 1 .	C	trace	o	o	+++	o	o	o	+++	C
C.S. 3 .	C	trace	o	o	+++	o	o	o	+++	C
C.S. 5 .	+++	o	o	o	+++	o	o	o	C	C
C.S. 7 .	C	+	o	o	+++	o	o	o	+++	C
C.S. 17 (1) .	C	trace	o	o	+++	o	o	o	+++	C
C.S. 21 .	+++	o	o	o	+	o	o	o	C	C
C.S. 27 .	C	o	o	o	+++	o	o	o	+++	C
A 3 .	+++	o	o	o	+++	o	o	o	C	C
A 6 .	C	++	o	o	C	o	o	o	+++	+++
A 9 .	C	++	o	o	C	o	o	o	+++	+++
A 15 .	C	+	o	o	C	o	o	o	+++	+++
A 17 .	+++	+	o	o	C	o	o	o	C	+++
B 2 .	C	o	o	o	+++	o	o	o	+++	C
A 7 .	C	C	C	C	C	C	C	+++	o	trace
C.S. 4 .	C	C	C	C	C	C	C	C	o	o
A 1 .	C	C	C	C	C	C	C	+	o	trace
B 12 .	C	C	C	C	C	C	C	+++	o	trace
*N 19 .	+++	o	o	o	+	o	o	o	C	C

* Naso-pharyngeal strain, see p. 231.

The quantities used were 10 mg. of culture per c.c. of 1-50 dilution of the serum and the test emulsions were the homologous strain A 17 and another main Group I strain, C.S. 2, of typical behaviour.

The results are very similar to those shown in Table IV. Differences in absorptive capacity between C and + + + coincide in some cases with similar differences in the former table, while in other cases absorptions indicated as + + + in Table IV here reach completeness. Such variations and coincidences may represent a tendency to sub-grouping within this main group, but, as has been noted above, they are not sufficiently regular to justify any such conclusion.

The object of inserting Table V is to show again the homogeneous nature of the main Group I, seen in Table IV; for this purpose the serum produced by A 17 was selected, as A 17 is one of the main group strains which showed the greatest tendency to diverge; further, A 17 is a strain of spinal origin, whereas P 1, used to produce the serum P 1, which has been chosen as the main Group I serum, was isolated from the naso-pharynx; yet the results with A 17 serum are practically indistinguishable.

Sub-Group (1).

In the next table (Table VI) absorption tests are given, using the same strains as in Table IV, but absorbing from the serum C.S. 8, produced by one of the strains which, on agglutination results, show relationship but certainly not identity with the main Group I. The technical details are exactly the same, and the masses of culture employed for absorption came from the same ascitic agar slopes as in the experiment summarised in Table IV; the symbols employed are also the same.

The results are equally sharp and definite. Absorption is complete with the three strains (including the homologue) which in Table I were seen to reach the full titre with this serum, namely, strains C.S. 8, A 7 and C.S. 20. On the other hand, the numerous strains of the main Group I, which agglutinated fairly strongly with serum C.S. 8, fail entirely, or almost entirely, to remove the agglutinin for either strain C.S. 8 or A 7, as also do the other strains which were shown to be related, but not identical with the main Group I.

Here, therefore, there is a group, which may be called the C.S. 8 group (sub-group (1) in Table III), quite as well defined apparently as the main Group I, the three strains composing it having on the results of absorption as much right to specific differentiation as the much larger group, although the agglutinogenic action of the type strain C.S. 8.

TABLE VI.

Absorption of agglutinin from serum C.S. 8.

Serum	Agglutination with Homologous Strain C.S. 8					Agglutination with Related Strain A 7					Absorption of Agglutinin for Homologous Strain C.S. 8	Absorption of Agglutinin for Strain A 7
	100 c	600 c	1000 c	1400 c	2000 ++	100 c	600 c	1000 c	1400 c	2000 ++		
Control un-absorbed												
Absorbed by Strain												
P 1 . .	c	c	+++	+	o	c	c	c	++	o	+	trace
C.S. 1 . .	c	c	c	++	o	c	c	c	+++	o	trace	trace
C.S. 2 . .	c	c	c	+++	+	c	c	c	+++	+	trace	trace
C.S. 3 . .	c	c	+++	++	o	c	c	c	+++	+	+	trace
C.S. 5 . .	c	c	+++	++	o	c	c	c	c	++	+	o
C.S. 6 . .	c	c	c	+++	+	c	c	c	c	++	trace	o
C.S. 7 . .	c	c	c	+++	+	c	c	c	c	++	trace	o
C.S. 17 (1) .	c	c	c	+++	+	c	c	c	c	++	trace	o
C.S. 18 . .	c	c	c	+++	+	c	c	c	+++	++	trace	trace
C.S. 21 . .	c	c	+++	+	o	c	c	c	+++	+	+	trace
C.S. 24 . .	c	c	c	+++	+	c	c	c	c	+	trace	o
C.S. 25 . .	c	c	c	+++	+	c	c	c	c	+	trace	o
A 3 . .	c	c	c	+++	+	c	c	c	c	++	trace	o
A 4 . .	c	c	c	c	+	c	c	c	c	+	o	o
A 6 . .	c	c	c	c	++	c	c	c	+++	+	o	trace
A 9 . .	c	c	c	+	o	c	c	+++	++	o	trace	+
A 11 . .	c	c	c	c	+	c	c	c	c	o	o	o
A 12 . .	c	c	c	c	+	c	c	c	c	++	o	o
A 14 . .	c	c	c	c	+	c	c	c	+++	+	o	trace
A 15 . .	c	c	c	c	++	c	c	c	c	+	o	o
A 18 . .	c	c	c	+++	+	c	c	c	+++	+	trace	trace
A 17 . .	c	c	c	c	++	c	c	c	c	++	o	o
B 2 . .	c	c	+++	+	o	c	c	c	+	+	+	trace
C.S. 8 . .	++	o	o	o	o	o	o	o	o	o	c	c
A 7 . .	+++	o	o	o	o	++	o	o	o	o	c	c
C.S. 20 . .	+	o	o	o	o	o	o	o	o	o	c	c
C.S. 4 . .	c	c	c	+++	+	c	c	c	c	++	trace	o
A 13 . .	c	c	c	++	+	c	c	c	c	++	trace	o
A 1 . .	c	c	c	+++	++	c	c	c	c	+	trace	o
A 2 . .	c	c	++	+	o	c	c	+++	+	o	+	+
A 16 . .	c	c	c	c	++	c	c	c	c	++	o	o
B 12 . .	c	c	c	+	+	c	c	c	+	o	trace	trace
B 3 . .	c	c	c	c	++	c	c	c	c	++	o	o
A 10 . .	c	c	c	++	+	c	c	c	c	+	trace	o
C.S. 14 . .	c	c	c	c	++	c	c	c	c	++	o	o

unlike the type strain of the main group, P 1, is less "pure," since its serum agglutinates many of the latter group to almost the full titre. The same overflow of the agglutinating action to strains not belonging to the group was observed in connection with serum A 17, which, as has just been seen, seems as pure a type of the main Group I as does C.S. 8 for the C.S. 8 group, when absorption is resorted to instead of agglutination as the test.

So striking was this selective action in the absorption test with serum C.S. 8 that further experiments were done in which very large amounts of culture were employed, 80 mg. in one c.c. of 1-100 dilution of serum, to see whether protein-complexes capable of absorbing agglutinin were present at all in these related but not identical strains. No increase in absorption appeared, although very marked agglutination with abundant deposit had occurred in the absorbing mixture; the serum still agglutinated completely up to 1-1500 after absorption with all the main Group I strains except two (A 9 and A 12), with which slight absorption had taken place, agglutination being complete only up to 1000. The agglutinin for the strains themselves contained in serum C.S. 8, in most cases sufficient to give complete agglutination at 1-1000, had, however, completely disappeared after absorption.

What explanation can be given for this cross-agglutination without cross-absorption in the case of these two strains and sera C.S. 8 and P. 1? The following hypothesis would appear to meet the case. The two special antigens are present in both the strains C.S. 8 and P. 1; in the former strain the amount of special P. 1 antigen is small and in the latter the amount of special C.S. 8 antigen is small. In the sera produced by the two strains both agglutinins are present, the P. 1 agglutinin being, however, less in amount in C.S. 8 serum and the C.S. 8 agglutinin less in amount in P. 1 serum, so that when each serum is diluted to the full titre the agglutinating action of the "foreign" agglutinin in each case disappears. In consequence when C.S. 8 serum is absorbed by the strain P. 1 only the P. 1 portion of its total agglutinin is removed; but this portion is in any case non-effective at the full dilution so that no diminution in the activity of the C.S. 8 agglutinin is visible after absorption by P. 1. Exactly the same happens when P. 1 serum is absorbed by strain C.S. 8.

There remain eight strains related to Group I on the strength of agglutination tests, but excluded by negative absorption tests from both the groups just discussed.

Sub-Group (2).

Serum A 13, produced by one of these, agglutinates one other strain to the full titre and one to 1-1000, while most of the main Group I and the sub-group (1) strains are agglutinated up to 1-100 only. Ab-

sorption tests with this serum are summarised in Table VII and amply confirm the suspected existence of still another group. The technical details and symbols employed are in general the same as in Tables IV, V and VI.

TABLE VII.

Absorption of agglutinin from serum A 13.

Serum	Agglutination with Homologous Strain A 13				Agglutination with Related Strain C.S. 4				Absorption of Agglutinin for Homologous Strain A 13	Absorption of Agglutinin for Strain C.S. 4
	100 c	300 c	800 c	1200 c	100 c	300 c	800 c	1200 c		
Control unabsorbed										
Absorbed by Strain										
C.S. 1 . .	c	c	c	+	c	c	c	+++	trace	o
C.S. 17 (1) .	c	c	+++	+	c	c	c	+	+	trace
C.S. 8 . .	c	c	c	c	c	c	c	c	o	o
C.S. 20 . .	c	c	c	++	c	c	+++	++	o	trace
C.S. 4 . .	+	o	o	o	o	o	o	o	c	c
A 13 . .	+	o	o	o	+	o	o	o	c	c
A 23 . .	c	+++	++	+	c	c	+++	+	++	+
A 1 . .	c	c	+++	+	c	c	++	+	+	+
A 2 . .	c	c	c	+	c	c	c	+	trace	trace
A 16 . .	c	c	+	o	c	c	o	o	+	+
A 10 . .	c	+	o	o	+++	o	o	o	+++	c
C.S. 16 . .	c	c	c	c	c	c	c	c	o	o
*N 29 . .	+	o	o	o	o	o	o	o	c	c

* Naso-pharyngeal strain, see p. 231.

It will be seen that, of the twelve spinal strains tested, two only, the homologous strain A 13 and strain C.S. 4, give complete absorption, each absorbing the agglutinin for both itself and the other. In addition, strain A 10 gives well-marked absorption of the agglutinin for A 13 and complete absorption of that for C.S. 4, while strain A 23 absorbs the greater part of the agglutinin for A 13. All the others, including strains identified as belonging to the main Group I, the sub-group (1), the main Group II, and others unidentified but related by agglutination to Group I, give more or less definite but slight absorption only.

The results of absorption from serum A 13 are thus less sharply defined than those of the sera just discussed, in the sense that allied but not identical strains exist which also absorb agglutinin, but it is evident that the strains A 13 and C.S. 4 have been identified with one another and sharply distinguished from the groups hitherto established; while two other strains, A 10 and A 23, not hitherto identified with

any group, have been placed in close relationship with this sub-group (2), of which A 13 is the type.

Sub-Group (3).

In Table VIII, which follows, the results are given of absorption from the serum prepared by inoculation of this A 10. The technique and symbols employed are as in previous absorption experiments.

The results obtained are remarkable. In the first place, as might have been expected, the related strains of the previous group A 13 and C.S. 4, as well as the strain A 23, which agglutinated to the full titre with serum A 10 (*vide* Table I), all absorb completely, or almost completely, the agglutinin for the homologous strain. This confirms the deduction drawn from Table VII, namely, the close relationship of these four strains. Of the other strains employed, C.S. 1, a typical main Group I strain, and A 1 and A 2, allied to but not identical with this main group, all fail to absorb more than small amounts of the A 10 agglutinin. A 1, however, absorbs a marked amount of the agglutinin for the strain A 13, as also does A 16, which is another unidentified ally of the main Group I. These two, then, though only distantly related to A 10, have a connecting relative in the strain A 13.

Strains C.S. 16, C.S. 14, and A 24, on the other hand, are definitely members of Group II, being agglutinated strongly by the main Group II serum and not at all by the main Group I, while the sera they produce by injection into animals agglutinate Group II strains to a high titre and Group I strains slightly, if at all. Yet C.S. 16 absorbs the A 10 agglutinin almost, if not quite, as well as the strains C.S. 4, A 13, and A 23, which have been put down as closely related to A 10, while A 24 absorbs only a small amount of A 10 agglutinin and C.S. 14 almost none.

This is a case in which agglutination reactions evidently fail to agree with the results of absorption, since strain C.S. 16 agglutinates with A 10 serum up to 1-500 only (*vide* Table I), a level which is reached also by most of the strains in Table VII which show feeble, if any, absorption.

For the present, until the absorption results with the Group II sera have been discussed, it will be better simply to note this anomaly, without trying to explain it. But it may be observed at this point that the sera P 1, A 17, and C.S. 8, behave very differently from serum A 10 in the sharpness with which absorption differentiates individual strains. The former three either show practically complete absorption or practically none, whereas A 10 serum gives all degrees of absorption with

TABLE VIII.

Absorption of agglutinin from serum A 10.

Serum	Agglutination with Homologous Strain A 10							Agglutination with Related Strain A 13							Absorption of Agglutinin for Homologous Strain A 10	Absorption of Agglutinin for Homologous Strain A 13
	100	300	600	800	1000	1500		100	300	600	800	1000	1500			
Control unabsorbed	C	C	C	C	C	+	+	C	C	C	C	C	+	+	+	+
Absorbed by Strain																
C.S. 1 . . .	C	C	C	C	+	+	+	C	C	C	C	+	0	+	+	+
C.S. 4 . . .	C	+	+	0	0	0	0	+	0	0	0	0	0	+	+	0
A 13 . . .	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A 23 . . .	+	0	0	0	0	0	0	C	+	+	0	0	0	+	+	+
A 1 . . .	C	C	C	C	+	+	+	C	+	+	+	0	0	+	+	+
A 2 . . .	C	C	C	C	+	+	+	C	C	C	+	+	+	+	+	+
A 16 . . .	C	C	C	+	0	0	0	C	+	+	+	+	+	+	+	+
A 10 . . .	0	0	0	0	0	0	0	+	0	0	0	0	0	+	+	+
C.S. 16 . . .	+	0	0	0	0	0	0	+	+	+	0	0	0	0	C?	0
C.S. 14 . . .	0	C	C	C	+	+	+	C	+	+	0	0	0	+	+	+
A 24 . . .	0	C	C	+	+	+	+	C	C	C	+	+	+	+	+	+
*N 4 . . .	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Naso-pharyngeal strain, see p. 231.

different strains. Further, as has been noted, in the case of the former sera increase in the amount of bacterial protein used for absorption does not increase the amount of agglutinin removed by the poor absorbers. With serum A 10 increase in the amount of culture to 80 mg. per c.c. of 1-50, or repeated addition of smaller amounts, increases the amount of absorption in the case of certain strains, *e.g.* A 24, but not in others, *e.g.* C.S. 14. This quality is characteristic of two of my Group II sera.

The strain A 10 is one of those given me by Dr Arkwright. It came from one of the first cases in the epidemic which broke out among Canadian troops in this country, and has been described already by Dr Arkwright (1915)¹ under the name of "Murray"; in his description he records that it belonged serologically to the meningococcus species, and not to the parameningococcus of Doptcr. Translated into the terms of my classification, this means that it belonged to Group I, and not to Group II. In my hands, although it inclines to the main Group I on its agglutination reactions, and is linked up to it by the relations shown by absorption, it has evidently affinities in addition which connect it with Group II.

There are thus several considerations all leading to the conclusion that A 10, whether originally or as the result of subsequent variation, lies intermediate between the main Group I and the main Group II, for

(1) it agglutinates equally, though relatively feebly, with the sera of both groups;

(2) it does not absorb agglutinin from either of the two main group sera, but

(3) the serum which it produces agglutinates indifferently, though weakly, strains of both main groups, and

(4) picks out, as agglutinating to high titre, a strain allied to Group I (A 13), and also one allied to Group II; while

(5) its agglutinin is absorbed both by strains allied to Group I and by at least one typical Group II strain; and

(6) the behaviour of the serum on absorption with different quantities of culture resembles that of other sera prepared with Group II strains.

Sub-Group (4).

Example of an aberrant type.

The absorption tests with serum A 2 may be summed up shortly without a table. None of the other spinal strains absorb more than small amounts of A 2 agglutinin, even when added in great excess, and the

¹ *Brit. Med. Journ.*, II. 885.

Group II strains in particular are completely devoid of absorptive capacity; indeed, after absorption with large amounts of these the titre of the "absorbed" serum appears slightly higher than before, the agglutination in the highest dilutions being more complete. Strain A 2, therefore, remains alone among my known pathogenic strains as the type of its sub-group, but it will be seen later that this sub-group is well represented among naso-pharyngeal meningococci.

As has been noted, it agglutinates indifferently with sera of both Group I and Group II, but to a low titre with both. It absorbs small amounts of agglutinin from certain Group I sera, but none, or practically none, from the Group II. It has, therefore, been put among the strains which lean towards the main Group I. With strain A 2 a serum of high titre was obtained after prolonged immunisation, agglutinating it completely at 1-1500. No other strain was agglutinated to the same level with this serum, but among spinal strains one, B 12, gave almost complete agglutination at 1-1000; two strains, C.S. 4 and A 13, which belong to one group, as has been seen, were agglutinated completely at 1-500, while C.S. 20, belonging to another group, and A 15, which was identified with the main Group I, almost reached the same titre. The others of the main Group I gave complete agglutination at 1-100, but only traces of agglutination at 1-500. The Group II strains were either completely negative, or gave only traces of agglutination at 1-100.

The agglutinogenic characters of A 2 thus confirm in the main its position as allied to Group I, but show that it possesses a pronounced individuality, distinguishing it from all the other spinal strains in my possession.

Absorption tests with serum A 2 confirm its peculiarities; none of the spinal strains, not even those agglutinating relatively well, remove any but the merest traces of the homologous agglutinin, although removing all the agglutinin for themselves. It must be noted further that the other strains hitherto unidentified, strains A 1, A 16, B 12, B 1, B 3, B 5 and B 10, fail to disclose definite relationship with A 2 as the result of application of its serum.

These strains may resemble A 2 in possessing serological properties peculiar to each, or they may belong to entirely different groups. It would be necessary, in order to settle more definitely their relationship, to investigate their agglutinogenic action, but even this, as has been seen in connection with A 2, and as will be seen with the naso-pharyngeal strains, might leave the question in doubt.

Sub-Group (5).

The three strains placed together in this sub-group are those which, though showing by their agglutination that they incline definitely towards the main Group I, all fail to absorb more than small amounts of agglutinin from the sera of this main group and the sub-group sera. Two of them, however, have been identified by absorption tests with a naso-pharyngeal strain which itself is similarly inclined to the main Group I, but also fails to absorb. This sub-group (5), then, seems to consist of two sub-groups, the second consisting solely of the strain B 12, which, like A 2, is highly individual in its characters.

Main Group II.

The results of absorption with the main Group II serum, P 2, as has been mentioned, have not the uniformity which is typical of the main Group I. In Table IX, subjoined, an example is given of the irregularity often met with. The quantity of culture employed in each case was 15 mg. for each c.c. of 1-50 dilution of the serum; this is the minimum for complete absorption of agglutinin by the homologous strain for itself, but does not reach the minimum which certain strains require to remove completely the agglutinin, either for themselves or for the homologous strain. Hence some of the strains in this table which fail to remove the agglutinin completely appear as incomplete absorbers only because, not 15 mg., but, say, 30 mg., of culture were required to complete the removal.

But it will be seen that those strains, numbers (1) to (7), which have already been identified with other groups are in agreement in removing traces at most of the homologous agglutinin. Similarly, four of the five strains which, in Table I, were shown to agglutinate feebly with all the sera, and were hence not capable of classification (Nos. 9, 10, 11, 12 here), fail to absorb definite amounts of agglutinin.

On the other hand, of the twenty strains put in the main Group II on the strength of agglutination, eight give absorption equal, or almost equal, to the homologous strain, nine give absorption just short of complete, while three give definite absorption but leave intact perhaps half the agglutinin for the homologous strain.

Of these three partial absorbers one agglutinates to full titre with serum P 2, while two agglutinate completely at 1-1500 (*vide* Table I).

Some indication of sub-grouping within Group II appears on inspection of the column recording absorption of agglutinin for C.S. 14.

TABLE IX.

Absorption of agglutinin from serum P 2.

Serum	Agglutination with Homologous Strain P 2					Agglutination with Related Strain C.S. 14					Absorption of Agglutinin for Homologous Strain P 2	Absorption of Agglutinin for Strain C.S. 14
	100	600	1000	1400	2000	100	600	1000	1400	2000		
Control unabsorbed	c	c	c	c	+++	c	c	c	c	+++		
Absorbed by Strain												
(1) C.S. 1 .	c	c	c	c	+	c	c	c	+++	o	trace?	+
(2) C.S. 17 (1)	c	c	c	c	+	c	c	c	+	o	trace?	+
(3) C.S. 25 .	c	c	c	c	+++	c	c	c	+++	o	o	+
(4) C.S. 8 .	c	c	c	c	+++	c	c	c	c	++	o	o
(5) C.S. 20 .	c	c	c	c	++	c	c	c	++	+	o	+
(6) C.S. 4 .	c	c	c	c	++	c	c	c	++	+	o	+
(7) A 23 .	c	c	c	++	+	c	c	++	+	o	+	++
(8) A 16 .	c	c	c	c	+	c	c	c	+++	o	trace?	+
(9) B 1 .	c	c	c	+++	+	c	c	++	+	o	+	+
(10) B 3 .	c	c	c	c	++	c	c	c	c	++	o	o
(11) B 5 .	c	c	c	c	+	c	c	c	o	o	trace?	+
(12) B 10 .	c	c	c	c	++	c	c	c	c	++	o	o
(13) A 10 .	c	c	c	+++	+	c	c	++	+	o	+	+
(14) A 24 .	c	+++	++	o	o	+	o	o	o	o	+++	c
(15) A 22 .	c	++	o	o	o	o	o	c	o	o	+++	c
(16) C.S. 9 .	c	+++	o	o	o	+++	o	o	o	o	+++	c
(17) C.S. 10 .	+++	+	o	o	o	o	o	o	o	o	c	c
(18) C.S. 11 .	c	++	o	o	o	o	o	o	o	o	+++	c
(19) C.S. 12 .	c	+	o	o	o	o	o	o	o	o	+++	c
(20) C.S. 14 .	c	c	+++	++	o	o	o	o	o	o	++	c
(21) C.S. 15 .	++	+	o	o	o	o	o	o	o	o	c	c
(22) C.S. 16 .	o	o	o	o	o	o	o	o	o	o	c	c
(23) C.S. 17 (2)	c	c	+++	++	+	c	c	c	+++	+	++	+
(24) C.S. 19 .	c	c	+++	+++	+	++	o	o	o	o	++	c
(25) C.S. 22 .	++	o	o	o	o	+++	o	o	o	o	c	c
(26) C.S. 26 .	+++	+	o	o	o	+	o	o	o	o	c	c
(27) A 20 .	+++	++	o	o	o	+	o	o	o	o	c	c
(28) A 25 .	c	c	+++	+	o	++	o	o	o	o	++	c
(29) B 4 .	c	+++	+	+	o	c	++	o	o	o	+++	+++
(30) B 6 .	c	+++	o	o	o	c	+	o	o	o	+++	+++
(31) B 7 .	+++	+	o	o	o	+++	+	o	o	o	c	c
(32) B 8 .	c	++	o	o	o	c	+	o	o	o	+++	+++
(33) B 9 .	++	+	o	o	o	c	+	o	o	o	c	+++
(34) B 11 .	c	++	o	o	o	c	+	o	o	o	+++	+++
(35) P 2 .	++	+	o	o	o	+	o	o	o	o	c	c

This strain is itself one of the partial absorbers although a good agglutinator; if, in serum P 2, the agglutinin responsible for the agglutination of C.S. 14 were in some way different from the main agglutinin produced by P 2, then strains which differed from P 2 in the same way as C.S. 14 does, ought to remove completely the agglutinin for C.S. 14, but to a varying and less extent for P 2. And, in fact, strain C.S. 19, which absorbs P 2 agglutinin only “++,” absorbs C.S. 14 agglutinin completely, while many of the strains absorbing P 2 just short of completely, remove all the agglutinin for C.S. 14.

The indication is that C.S. 14 is allied to C.S. 19, and that both differ slightly from P 2 and most of the other strains, but the evidence is slender, as will be seen later.

One strain removes agglutinin only partially for both C.S. 14 and P 2; this is C.S. 17 (2), the variant of the Group I strain, C.S. 17, already referred to as having changed its character. One strain, B 8, which, on agglutination, appeared a doubtful adherent of Group II, is shown by absorption to be closely allied to, if not identical with, the main group.

The absorptive action of strains C.S. 14, C.S. 16 and A 24 should be specially noted, as sera have been prepared with them, the behaviour of which will be recorded below. Of these C.S. 16 and A 24 are good absorbers, while C.S. 14, as just remarked, is a partial absorber.

In Table X are given the results of absorption from serum C.S. 14, the quantity of culture used being 10 mg. per c.c. of 1-50 serum dilution.

In the first place it may be noted that the first five strains, which belong to Group I, absorb, at most, traces of agglutinin, as do also the next two strains, B 1 and B 3, which were doubtful adherents of Group II. Strain A 10, the strain intermediate between the two groups, also removes only a minute amount of C.S. 14 agglutinin.

On the other hand fifteen strains, which by agglutination were put in Group II, absorb all or almost all the agglutinin both for C.S. 14 and for P 2, the type strain of Group II. Among these are C.S. 19 and C.S. 17 (2) which showed only partial absorption with P 2 serum (*vide* Table IX).

No well-marked difference can be detected between the absorption of agglutinin for the homologous strain C.S. 14 and that for the type strain P 2.

In Table XI are given the results of absorption from serum C.S. 16 by a selection of Group II strains, with, as controls, three strains more

closely allied to Group I and the four strains which, on agglutination, were referable to neither group but inclined, perhaps, to Group II.

It will be seen that all these controls absorb, at most, traces of agglutinin for both the test emulsions, while the strains already put in Group II absorb the agglutinin for both, either completely or almost completely. It is worth noting that strain A 10 which, as was seen in

TABLE X.

Absorption of agglutinin from serum C.S. 14.

Serum	Agglutination with Homologous Strain C.S. 14				Agglutination with Related Strain P 2				Absorption of Agglutinin for Homologous Strain C.S. 14	Absorption of Agglutinin for Strain P 2
	100 C	500 C	1000 C	1500 C	100 C	500 C	1000 C	1500 C		
Control unabsorbed										
Absorbed by Strain										
C.S. 1 . . .	C	C	C	++	C	C	C	+++	trace	trace?
C.S. 17 (1) . .	C	C	C	C	C	C	C	C	o	o
C.S. 8 . . .	C	C	C	C	C	C	C	C	o	o
C.S. 4 . . .	C	C	C	+++	C	C	C	++	trace?	trace
A 23 . . .	C	C	+++	+	C	C	C	C	+	o
B 1 . . .	C	C	+++	+	C	C	++	+	+	+
B 3 . . .	C	C	C	C	C	C	C	+++	o	trace?
A 10 . . .	C	C	+++	+	C	C	C	C	+	o
A 24 . . .	o	o	o	o	o	o	o	o	C	C
A 22 . . .	+	o	o	o	o	o	o	o	C	C
C.S. 9 . . .	+	o	o	o	o	o	o	o	C	C
C.S. 10 . . .	++	o	o	o	C	o	o	o	C	+++
C.S. 11 . . .	o	o	o	o	o	o	o	o	C	C
C.S. 12 . . .	+++	+++	+	o	++	+	o	o	C?	C
C.S. 14 . . .	o	o	o	o	+	o	o	o	C	C
C.S. 15 . . .	+	o	o	o	o	o	o	o	C	C
C.S. 16 . . .	++	o	o	o	o	o	o	o	C	C
C.S. 17 (2) . .	C	+	o	o	+++	o	o	o	+++	C
C.S. 19 . . .	+++	o	o	o	o	o	o	o	C	C
C.S. 22 . . .	+	o	o	o	o	o	o	o	C	C
C.S. 26 . . .	+	o	o	o	o	o	o	o	C	C
A 20 . . .	o	o	o	o	o	o	o	o	C	C
A 25 . . .	o	o	o	o	o	o	o	o	C	C
P 2 . . .	o	o	o	o	o	o	o	o	C	C

Table VIII, produced a serum from which C.S. 16 absorbed practically all the agglutinin, fails entirely to absorb agglutinin from C.S. 16 serum; on repeating this absorption test with A 10 on C.S. 16 serum, but using 60 mg. of culture per c.c. in three instalments of 20 mg. instead of 10 mg. in all, the negative result persisted, as was the case also with strains C.S. 20 and A 13 similarly tested.

Sub-Group (6).

Finally the results of absorption of agglutinin from serum A 24 may be given (Table XII). Strain A 24, as has been remarked (*vide* Tables I, IX, X, and XI), agglutinated to the full titre with all the Group II sera and absorbed their agglutinin completely, but the agglutination reactions of the serum it produced indicated that it possessed peculiar characters,

TABLE XI.
Absorption of agglutinin from serum C.S. 16.

Serum	Agglutination with Homologous Strain C.S. 16				Agglutination with Related Strain A 22				Absorption of Agglutinin for Homologous Strain C.S. 16	Absorption of Agglutinin for Strain A 22
	100 C	400 C	800 C	1200 C	100 C	400 C	800 C	1200 C		
Control unabsorbed										
Absorbed by Strain										
C.S. 20 .	C	C	C	C	C	C	C	C	O	O
A 13 .	C	C	C	C	C	C	C	C	O	O
A 10 .	C	C	C	C	C	C	C	C	O	O
A 24 .	+++	+	O	O	++	+	O	O	C	C
C.S. 10 .	+++	+	O	O	+	O	O	O	C	C
C.S. 14 .	+++	+	O	O	++	O	O	O	C	C
C.S. 16 .	++	O	O	O	+	O	O	O	C	C
C.S. 19 .	+++	+	O	O	++	O	O	O	C	C
A 20 .	+++	+	O	O	+++	O	O	O	C	C
A 25 .	C	+++	O	O	+++	+	O	O	+++	C
B 1 .	C	C	C	+++	C	C	C	C	trace?	O
B 3 .	C	C	C	C	C	C	C	+++	O	trace?
B 4 .	+++	O	O	O	+	O	O	O	C	C
B 5 .	C	C	C	+++	C	C	C	C	trace?	O
B 6 .	C	O	O	O	+	O	O	O	+++	C
B 7 .	+++	O	O	O	+	O	O	O	C	C
B 8 .	O	O	O	O	trace	O	O	O	C	C
B 9 .	C	O	O	O	+	O	O	O	+++	C
B 10 .	C	C	C	C	C	C	C	C	O	O
B 11 .	C	O	O	O	+	O	O	O	+++	C

not revealed by these sera, since only one of the other Group II strains appeared to be specifically affected.

The technical details of the experiment are as before, but the symbols have been given a higher value in consequence of the low titre of the serum, complete absorption being presumably easier from a weak serum.

The peculiar characters of A 24 and A 22 are confirmed by the absorption test and justify the position of these two strains as forming

a sub-group within the main Group II. The strain A 22, which, as was shown in Table I, was unique in reaching the full titre for this serum, removes, as might be expected, all the agglutinin for A 24, while one other strain; C.S. 11, which agglutinated with A 24 serum up to 1-500 only, absorbs almost all the agglutinin for both A 24 and A 22. Strain C.S. 9, which also agglutinated up to 1-500, absorbs perhaps half the agglutinin, while C.S. 22, which had the same agglutination titre, absorbs none of the A 24 agglutinin and only a small amount of that for A 22.

The type strain, P 2, absorbs none of the A 24 agglutinin, in spite of the fact that A 24 absorbs the P 2 agglutinin from P 2 serum almost completely.

TABLE XII.

Absorption of agglutinin from serum A 24.

Serum	Agglutination with Homologous Strain A 24				Agglutination with Related Strain A 22				Absorption of Agglu- tinin for Homo- logous Strain A 24	Absorp- tion of Agglu- tinin for Strain A 22
	100 c	400 c	800 c	1000 c	100 c	400 c	800 c	1000 c		
Control un- absorbed by Strain										
C.S. 9 . .	+++	+++	++	c	c	+++	o	o	++	++
C.S. 11 . .	++	trace	o	o	++	o	o	o	c	c
C.S. 22 . .	c	c	c	c	c	+++	o	o	o	++
C.S. 14 . .	c	c	c	c	c	+++	trace	o	o	++
A 22 . .	o	o	o	o	o	o	o	o	c	c
A 24 . .	o	o	o	o	o	o	o	o	c	c
A 25 . .	c	c	+++	+++	c	+++	o	o	+	++
P 2 . .	c	c	c	c	c	c	+++	o	o	+

Sub-Group (7).

The sub-group (7) which was created on the strength of agglutination results and recorded in Table III has by the use of the absorption test had its numbers reduced from five to four, since the strain B 8 although agglutinating relatively feebly has been shown to absorb all the agglutinin from serum C.S. 16. The other four strains, however, remain as a sort of scrap-heap since though they certainly agglutinate rather better with the Group II sera than with Group I yet their agglutination can in no case be said to be decisive, and they absorb practically none of the various agglutinins tested. They may belong to one group or may each represent strains of high individuality; they may be compared in this respect to the strain A 2 and may bear the same relation to the main Group II that this strain has shown towards the main Group I.

GENERAL BEHAVIOUR OF GROUP II SERA ON ABSORPTION.

The results of absorption from the Group II sera are evidently much more complicated and difficult to explain than those with the Group I sera.

In the first place there is the phenomenon, which was noted in connection with serum A 10, that increase in the amount of culture used for absorption may transfer a strain from the category of poor absorbers into that of complete absorbers. This is even more marked in the case of the Group II sera P 2 and C.S. 14. For example, with serum P 2 in one absorption experiment, using 8 mg. of culture per c.c. of 1-50 dilution, the homologous strain P 2 and strains C.S. 16, A 22, C.S. 22, out of fifteen Group II strains, were the only ones which reduced the titre to just below 1-500; with C.S. 9, C.S. 10, C.S. 11, C.S. 12, A 20, A 24, A 25, C.S. 14 and C.S. 19 the titre was reduced to incomplete at 1-1000 only; on repeating the extraction with the same amount of culture on the partially exhausted serum, the titre was reduced somewhat below the levels shown in each case in Table IX, *e.g.*, with P 2, the homologous strain, only traces of agglutination persisted at 1-100, as also with A 22, C.S. 22, C.S. 10, C.S. 11, C.S. 12 and A 20, while with C.S. 9, A 24, A 25 and C.S. 14 agglutination was still complete at 1-100 but incomplete at 1-500. If an arbitrary point had been chosen as determining a positive result, as, for example, complete or incomplete agglutination at 1-500, this dilution only being tested, and if the test had been performed only after the first extraction, very definite grouping might have appeared, P 2, C.S. 16, A 22, C.S. 22 forming one group among the Group II strains, while the others might have been regarded as doubtful allies. When more opportunity for absorption was provided by increasing the amount of extracting material, the results, as shown in Table IX, indicate that many more strains are capable of removing completely, or almost completely, the homologous agglutinin.

Exactly similar results have been obtained with serum C.S. 14; as with serum P 2, increase in the amount of culture used in the absorption test broadened the selective action of the agglutinin to a quite remarkable extent.

This behaviour makes it extremely difficult to use the absorption test in estimating the relationships of the different strains of Group II. Some indication of the presence of sub-groups is to be found in the relative ease with which certain strains remove agglutinin as compared with others, but it is very doubtful if any stress can be laid on this, since, if all the agglutinin in the test serum can be combined and removed by a strain, even though large quantities of culture are necessary, the negative controls remaining unaffected, it shows, at least, that the partial absorption given by the smaller amount does not depend on the presence in the serum of an anti-body which fits the homologous and identical strains but does not fit those absorbing less readily. And the demonstration of an anti-body which is incapable of absorption by non-identical strains is the admitted criterion in distinguishing "specific" from "group" agglutination.

Yet the existence in the Group II sera of such "group" agglutinins would seem to be demanded by the fact that they agglutinate and are absorbed to a small extent by strains such as those of the main Group I and some of its allies. The difference is that increase in the amount of culture used does not lead with these, as with the less active absorbers among Group II strains, to increased absorption. These strains which fail to absorb even when present in great excess act as controls to show that

in the action of large amounts of culture physical non-specific destruction of agglutinin is not responsible for absorption.

On what, then, depends the less easy absorption by strains which, in actual biochemical constitution, are apparently identical with the strain producing the serum?

To answer this would require a much more profound knowledge than is in existence of the factors at work in the absorption test, and it has not been found practicable at this time to investigate these factors as a problem apart from the classification of pathogenic meningococci and the identification of naso-pharyngeal strains with them.

The following considerations may apply. The absorption of agglutinin by a suspension of bacteria may be conceived as a simple chemical combination, each coccus, for example, being capable of combining with a certain definite maximum of the protein in the serum which carries the agglutinating property. This maximum might vary in different strains, so that a strain in which it was high would remove more agglutinin for a given number of cocci than another in which it was low, although the qualitative nature of the chemical action taking place did not differ. This supposition would account for most of the phenomena in the absorption tests with Group II strains, were it not for the difficulty caused by the fact that a strain may have a low maximum with one serum and a high maximum with another, though capable of removing all agglutinin from both.

An alternative conception depends on the phenomena observed in biological precipitation. When, for example, dilute horse serum is mixed with the serum of a rabbit immunized against horse protein, a bulky precipitate is thrown down, by far the greater part of which is derived, not from the dilute horse serum, the precipitinogen, but from the anti-serum, the precipitin. The same observation has been made with bacterial precipitation, such as occurs when a filtered extract of bacteria has been brought in contact with the corresponding anti-serum. Here the amount of the bacterial protein has no direct relation with the amount of protein thrown out of solution and removed from the anti-serum.

There is considerable evidence that bacterial precipitin and bacterial agglutinin are attached to one and the same protein in the anti-serum, but, even if attached to different proteins, the production of a precipitin-precipitum in the mixture would necessarily produce a mechanical agglomeration and deposit of the suspended cocci which would both simulate and take part in the total agglutination. Hence, supposing, as seems reasonable, that dissolved bacterial proteins, as well as intact cocci, are present in the suspensions, then not only agglutination but removal of agglutinin would depend both on deposition of cocci which had combined with agglutinin and on the precipitation of large quantities of anti-body in consequence of the presence of dissolved bacterial protein acting as a precipitinogen.

Hence one might account for the different behaviour of Group I and Group II strains in absorption tests by supposing that Group I strains more readily liberate their protein into solution, so that more of this second method of removing anti-body occurs; while Group II strains might depend for agglutination and for agglutinin-absorption on the much less powerful action of direct combination of cocci with agglutinin.

I have some evidence that suspensions of those strains which remove agglutinin on the addition of small amounts of culture contain more detached soluble protein than those which require large amounts, since I have repeatedly seen with Group I

strains, as contrasted with Group II strains in which the phenomenon is much less marked, that when a suspension of culture has been freed from all intact cocci by the centrifuge or prolonged standing, or by filtration, the clear liquid produces almost as large a precipitum with the anti-serum as the same fluid containing the suspended cocci; this precipitum must consist chiefly of the anti-body present in the serum, the removal of which probably depends on the precipitinogen contained in the dissolved bacterial protein and is thus governed by laws different from those ruling the absorption by intact cocci.

On this hypothesis, then, the different behaviour of certain strains and sera as regards the facility of absorption would be explained by the varying amount of dissolved protein present in the suspensions—(this would explain also the difference between individual Group II strains). A correlated factor may be the varying extent to which anti-body capable of behaving like precipitin is present in the serum—(this would still further explain the difference in behaviour between sera which respond to increase of culture by increase of absorption and those which do not).

But in reviewing generally the difficulties in reconciling the different behaviour of meningococcus strains and anti-sera, I can only repeat that, until the essential nature is known of the physico-chemical action which results in absorption of agglutinin, there is little probability of satisfactorily explaining the apparent anomalies.

Two of these anomalies, which have been specifically mentioned and are of similar character, are (1) the fact that strain A 24 absorbs completely from serum C.S. 14 the agglutinin for C.S. 14, while C.S. 14 removes none of the specific agglutinin for A 24 from serum A 24, and (2) that strain C.S. 16 removes all, or nearly all, the specific agglutinin for strain A 10 from serum A 10, but strain A 10 removes little or none from serum C.S. 16.

It is just possible that the specific agglutinin in A 24 serum consists almost entirely of very specialised anti-bodies, corresponding to very specialised antigens in strain A 24 and hence not affected by strain C.S. 14, while in serum C.S. 14 the anti-bodies present are of a more general character, each antigen in the strain having exerted an approximately equal effect on the animal. In the absorption of serum C.S. 14 by A 24 the antigens of general Group II character alone come into action, and the more special C.S. 14 anti-bodies, if any, are in such small amount that, though left in the serum after absorption by A 24, they only produce agglutination of low titre for C.S. 14.

A similar explanation in the other case meets with the difficulty that serum A 10 undoubtedly contains very special anti-bodies of predominating Group I character as well as the more general, and it is hard to conceive that strain C.S. 16 which, agglutinogenically, had little relation to A 10, contained enough of the special corresponding antigen to affect these appreciably, whereas serum C.S. 16 resembles very much serum C.S. 14 and would, therefore, contain special anti-bodies in less amount than the more general anti-bodies; it should hence be affected appreciably by contact with the general antigens of A 10.

The only explanation possible is that strain C.S. 16 at the time it was used for absorption had become modified in character, acquiring some of the characters more typical of A 10. That such modification may occur is to my mind probable, and the following section records variations in specific character as determined by absorption tests.

Variations in absorptive quality and capacity.

Variations in agglutinability have already been noted and illustrated in Table II. In particular, one strain, C.S. 17, was stated to have changed from agglutination to full titre with Group I serum when first isolated to agglutination to full titre with Group II serum after keeping in culture for a month, the other group serum in each case being almost if not quite negative. One month later it again agglutinated to full titre with the Group I serum and only slightly with Group II. This latter condition has persisted unchanged now for several months, but another sub-culture from that which gave Group II agglutination has remained equally definitely of Group II character in agglutination; the former I have designated C.S. 17 (1), the latter C.S. 17 (2). Both have been plated out at intervals of about a month and have given on each of three occasions practically pure cultures of their own agglutinating type, four colonies being investigated from each plate. One colony from C.S. 17 (2), however, agglutinated to 1-500 with serum P 1, the Group I serum, as well as to full titre with serum P 2, the Group II serum, and therefore might be regarded as tending towards an intermediate position.

From the Group I sera P 1 and A 17 the strain C.S. 17 (1) absorbs practically all the agglutinin for the homologous strains as for itself, while C.S. 17 (2) removes none either for the homologous strains or for C.S. 17 (1). From the Group II sera P 2 and C.S. 14 the strain C.S. 17 (1) absorbs no agglutinin either for the homologous strains or for C.S. 17 (2), whereas the latter absorbs rather less than half the agglutinin for the homologous strains (noted as + +), but all the agglutinin for itself.

From serum C.S. 8 both C.S. 17 (1) and C.S. 17 (2) remove no agglutinin for the homologous strain, but C.S. 17 (1) removes all the agglutinin for itself (titre before absorption 1-500), while C.S. 17 (2) leaves the agglutinin for C.S. 17 (1) intact.

From serum A 10, C.S. 17 (1) removes agglutinin entirely for itself, and "+ +" for the homologous strain, while C.S. 17 (2) removes none of either agglutinin.

The absorption tests thus confirm the Group I condition of C.S. 17 (1), but leave doubtful the exact position of C.S. 17 (2): yet by agglutination this strain is definitely of Group II, and this is confirmed by its agglutinogenic action, since a serum prepared with C.S. 17 (2) agglutinated all the Group II and left practically unaffected the Group I strains.

The conclusion to be drawn is either that this strain C.S. 17 changed its serological character entirely during cultivation, or that in the

original infection two strains of meningococci were present, one or other predominating at different times during cultivation until in separate instances one or other died out entirely. On the whole I feel inclined to take the latter view, but have felt bound to record the occurrence as a possible instance of profound variation in the serological character of a meningococcus strain.

Strain A 24 has been particularly variable in the extent of its absorbing powers for certain sera. At one time 10 mg. of its culture removed only traces of the homologous agglutinin from serum P 2, while three months later the same quantity removed all the homologous agglutinin, although in the two cases the cultures were equally well agglutinated by serum P 2. But even with its own serum such irregularities in absorptive power appeared. Sub-cultures from two colonies on the same plate differed entirely in their absorptive power, one removing all the A 24 agglutinin, while the other barely affected this serum, although again both agglutinated well. On the other hand, with serum C.S. 16, these two colonies, though differing markedly in agglutinability, one being complete at 1-1500, while the other did not agglutinate above 1-100, absorbed the C.S. 16 agglutinin completely in both instances.

Strain C.S. 16 absorbed from serum A 10 on November 11, 1916, only traces of agglutinin, whereas later, on February 21, 1917, it absorbed A 10 serum completely, although used in exactly the same amount and under the same conditions.

Finally, the strain C.S. 10 may be mentioned. In July, 1916, it absorbed C.S. 14 serum completely as it did again in August, but in November it had become much less agglutinable and also failed entirely to absorb C.S. 14 agglutinin. Since then it has remained inagglutinable with all the sera tested and removes no agglutinin from any of the Group II sera. Morphologically and culturally it is unchanged.

Explanation of Serological Differences.

Before proceeding to discuss the naso-pharyngeal strains it may be well at this point to summarise my argument as to the reason for the differences found by serological tests in different strains of meningococci obtained from spinal fluid, leaving over for the moment the question of the validity of these differences as criteria for classification into fixed types.

I have concluded, then, that peculiarities in the protein molecule affect the antigenic action of the different strains and excite in the

immunised animal the production of anti-bodies of similar peculiarity, that in some strains, *e.g.* those within or allied to Group I, this particularisation of the protein molecule is much more advanced than in others, *e.g.* Group II; in the former, serological identity is much more striking when it appears and serological difference is similarly more obvious, while in the latter the peculiar properties are in the background, and the general action of the protein as antigen and combining factor in serological reactions is more pronounced, so that peculiarities in the strains belonging to this group are readily concealed by the interaction of their more general characters. It is probable that peculiarities limited to small sub-groups of strains, or even to individuals, also exist in Group II, but, as a result of this quantitative difference in particularisation, the sub-groups among the former are well marked, while in the latter they are submerged in the general character of the large group¹.

These conclusions certainly seem to explain the experimental facts. The question remains whether these peculiarities are permanent features of the strains or only temporary, however strong they may be. Possibly they have been acquired during a long process of evolution, and hence deserve consideration as stages in the direction of specific differentiation; or they perhaps merely express variations recently acquired as the result of interaction with the tissues of their host.

Unfortunately, conclusive evidence on these points is extremely difficult, if not impossible, to get. In artificial culture the variations which I have been able to demonstrate are not conclusive evidence that in nature change can take place from one group to another, and are open to the objection that the original strain may not have been pure but contained two varieties existing side by side, but predominating at different times.

The relative proportions of the different types isolated from the human host in health and disease are, however, somewhat suggestive of modification due to environment, and this question will be referred to again when the serological reactions of naso-pharyngeal strains have been discussed.

NASO-PHARYNGEAL STRAINS.

As has already been indicated, these strains were isolated from naso-pharyngeal mucus, and each represents a colony found on plates of

¹ This difference between the groups may depend, however, on the accident of choice of strains used for producing sera; it seems possible that, with a different selection, sera of Group I might be found containing agglutinins in large amounts for its sub-groups, and sera of Group II (*cf.* serum A 24) containing agglutinins acting only on small sub-groups.

Kutscher's medium inoculated with this material. Morphologically and culturally they were indistinguishable from the meningococci isolated from cerebro-spinal fluid in cases of the specific disease. Sixteen represent the survivors of the thirty strains from Lambeth outpatients discussed in my former report; the remainder, making up the total of seventy-one, were isolated during the first half of 1916, chiefly from soldiers in camp or garrison.

Agglutination and Absorption Reactions.

In the following table (Table XIII) the dilutions are given at which complete agglutination was produced by the two sera, C.S. 14 and C.S. 16, with all the naso-pharyngeal strains in my possession. These sera were both prepared with strains of pathogenic origin, both belonging to Group II; the titre for the homologous strain was in each case 1-1500. In the same table the results of absorption of the agglutinin for the homologous strains are given for these two sera, using 10 mg. of culture with each strain for 1 c.c. of the serum diluted 1-50; the symbols have the same value as in the previous absorption tables for these sera (Tables X and XI).

It will be seen that, as with the spinal strains, the two sera have much the same agglutinating properties, the differences observed being of a minor quantitative character. Taken together they agglutinate to the full titre 30 of the 71 strains, while nine others are agglutinated to 1-1000 and five to 1-500 with one or other serum. This leaves 27 strains which on agglutination results are excluded from the group represented by C.S. 14 and C.S. 16, and already defined as Group II.

The results of absorption are closely comparable: 37 strains remove completely the agglutinin for the homologous strain from one or both the sera, while three others remove it almost completely, the effect being noted as + + + which means, as before, that agglutination of the homologous strain by the serum after absorption was complete at 1-100 but incomplete at 1-500. These 40 strains include the 30 reaching the full agglutination titre, the nine reaching 1-1000 and one of the five reaching 1-500.

With the remaining 31 strains absorption was either entirely negative (seven strains) or reduced the titre of the serum only to a slight degree.

The conspicuous feature of the table is thus the large number of strains, 56 per cent., which can be identified with one or both of the two strains of spinal origin, this identification depending on the combining properties of the strains in question with the anti-bodies produced by

TABLE XIII.

Naso-pharyngeal strains: results of agglutination and absorption with two Group II spinal sera.

Strain	Agglutination with Serum C.S. 14	Agglutination with Serum C.S. 16	Absorption from Serum C.S. 14	Absorption from Serum C.S. 16	Strain	Agglutination with Serum C.S. 14	Agglutination with Serum C.S. 16	Absorption from Serum C.S. 14	Absorption from Serum C.S. 16
N 1	100	100	tr.	tr.	N 37	100	500	o	+
N 2	tr.	tr.	tr.	tr.	N 38	1500	1500	c	c
N 3	tr.	100	tr.	tr.	N 39	1500	1500	c	c
N 4	100	100	+	tr.	N 40	1500	1000	c	c
N 5	100	500	+	+	N 41	1500	100	c	c
N 6	1500	1000	c	c	N 42	1000	1000	+++	c
N 7	tr.	100	tr.	tr.	N 43	1500	1500	c	c
N 8	o	100	tr.	tr.	N 44	1500	1500	+++	+++
N 9	1500	1000	c	+++	N 45	1500	1500	c	c
N 10	tr.	100	tr.	o	N 46	1500	1500	c	
N 11	tr.	o	o	o	N 47	1000	1500	+++	c
N 12	tr.	100	o	o	N 48	100	100	+	o
N 13	100	100	++	+	N 49	100	100	o	++
N 14	tr.	o	o	o	N 50	1000	1000	c	c
N 15	1500	100	+++	++	N 51	1500	1500	c	c
N 16	tr.	100	++	+	N 52	1000	1000	c	c
N 17	500	1500	+++	+	N 53	o	o		o
N 18	100	100	+	o	N 54	1000	1500	c	
N 19	o	o	o	o	N 55	1500	1500	+++	c
N 20	100	100	tr.	o	N 56	1000	1500	c	+++
N 21	1500	1000	c	+++	N 57	1500	1500	c	
N 22	1000	1500	c	c	N 58	o	100	tr.	+
N 23	tr.	o	tr.	tr.	N 59	1500	500	c	c
N 24	100	100	+		N 60	1500	1500	c	c
N 25	1500	1500	c		N 61	1500	1500	c	c
N 26	100	o	tr.	+	N 62	1000		c	
N 27	1500	1000	c		N 63	1000	1000	c	c
N 28	1500	1000	c	+++	N 64	o	o	o	
N 29	o	o	o	o	N 65	100	100	tr.	
N 30	100	o	o	+	N 66	500	500	o	tr.
N 31	1000	1000	c		N 67	100	100	o	tr.
N 32	1500	1500	c	c	N 68	o	500	o	tr.
N 33	1500	1500	c	c	N 69	500	500	c	c
N 34	1000	1000	c	c	N 70	1500	1000	c	c
N 35	1000	1000	c	c	N 71	1000		c	
N 36	500	1000	c	c					

the known pathogenic meningococci. This predominance of Group II strains applies both to contacts and non-contacts, and in this connection it is of great interest to remember that in the collection of 60 known pathogenic strains only 21, 35 per cent., were identified with this group,

while 33, 56 per cent., were either identified with, or shown to be nearly related to Group I.

There remain among the 71 naso-pharyngeal strains 31 which have still to be identified.

Among these, ten have been specifically identified with spinal strains belonging to Group I and its immediate allies, while six strains have given evidence of relationship but not identity. Table XIV summarises the reasons for the specific identification or relationship of these Group I naso-pharyngeal strains under the headings of (1) agglutination with sera prepared with spinal strains of the group, (2) absorption of agglutinin from these sera, (3) agglutinogenic action in the case of eight strains showing that the sera produced by them agglutinate certain spinal strains, and (4) absorption of agglutinin from these sera by these spinal strains. Information on the last two points has not been collected in the case of all strains, since the labour involved in preparing and testing sera with all would probably not have given results of proportionate value.

The sub-groups referred to in the column headed "*Absorption of Group I agglutinin and consequent sub-grouping*" are those found among spinal strains and recorded in Table III.

On examining this table it will be seen that only one strain, N 19, has been found identical with the main Group I, one strain, N 1, with the sub-group (1) represented by C.S. 8, one strain, N 29, with the sub-group (2), of which A 13 is the type, three strains, N 4, N 5, and N 13, with the closely-related sub-group (3), of which A 10 is the type spinal strain, while four, N 2, N 10, N 58, and N 67, have been identified with the sub-group (4), represented by strain A 2, which, though allied to the main Group I, is highly specialised, and has no near relative among my other spinal strains. One strain, N 48, is of much interest as being closely related to, if not identical with, four of the spinal strains which could not be identified by the use of any of the spinal sera, though two of them possessed definitely stronger affinities for Group I, and were hence put in sub-group (5), while two were indifferent to both the main group sera, and were put in sub-group (7), the "scrap-heap." In addition four strains, N 3, N 7, N 8, and N 11, have been shown to possess relationship with the spinal strain A 2, but on evidence insufficient for the presumption of specific identity. One of these, N 7, is similarly related to at least one of the main Group I strains, A 12, the relationship in common being apparently some quality of a minor nature in the case of A 12, since it is insufficient to differentiate A 12 from others of the main group.

TABLE XIV.

Serological relationship and classification of naso-pharyngeal strains allied to Group I.

Agglutinogenic Properties, i.e. Reactions of Serum prepared with Strain								
Strain	Agglutination with Group I Sera		Absorption of Group I Agglutinin and consequent sub-grouping		Agglutination of Spinal Strains			
					Absorption with same Spinal Strains			
N 1	Serum P 1,	500	Serum P 1,	+	[Full Titre, 1500]			
	„ C.S. 8,	1000	„ C.S. 8,	c	Main Group I,	100	+	
			Sub-group (1)		C.S. 8 Group,	1000	c	
					Main Group II,	o	o	
N 2	Serum P 1,	100	Serum P 1,	o	[Full Titre, 1000]			
	„ C.S. 8,	tr.	„ C.S. 8,	o	Main Group I,	tr.	o	
	„ A 2,	800	„ A 2,	c	C.S. 8 Group,	tr.	o	
			Sub-group (4)		A 2,	600	c	
N 3	Serum P 1,	tr.	Serum P 1,	o	<i>Not tested</i>			<i>Not tested</i>
	„ C.S. 8,	o	„ C.S. 8,	o				
	„ A 2,	500	„ A 2,	++				
			Related to sub-group (4)					
N 4	Serum P 1,	500	Serum P 1,	o	„		„	
	Sera C.S. 8 and A 2,	100	Sera C.S. 8 and A 2,	o				
	„ A 13 and A 10,	1000	„ A 13 and A 10,	c				
			Sub-group (3)					
N 5	Serum P 1,	100	Serum P 1,	tr.	„		„	
	„ A 10,	500	„ A 10,	+++				
N 7	Serum P 1,	500	Serum P 1,	o	[Full Titre, 800]			
	„ A 2,	500	„ A 2,	+	Main Group I,	300	tr. [A 12 = + + +]	
			Related to sub-group (4)		A 2,	800	++	
					Group II,	300	o	
N 8	Serum P 1,	500	Serum P 1,	o	<i>Not tested</i>			<i>Not tested</i>
	„ A 2,	500	„ A 2,	+				
N 10	Serum P 1,	500	Serum P 1,	++	[Full Titre, 1000]			
	„ C.S. 8,	o	„ C.S. 8,	o	Main Group I,	500	+	
	„ A 2,	500	„ A 2,	+++	Group C.S. 8,	tr.	o	
			Sub-group (4)		A 2,	1000	+++	
N 11	Serum P 1,	100	Serum P 1,	o	<i>Not tested</i>			<i>Not tested</i>
	„ C.S. 8,	100	„ C.S. 8,	o				
	„ A 2,	500	„ A 2,	+				
			Related to sub-group (4)					
N 13	Serum P 1,	tr.	Serum P 1,	o	[Full Titre, 1500]			
	„ C.S. 8,	500	„ C.S. 8,	+	Main Group I,	500	o	
	„ A 10,	500	„ A 10,	+++	Group C.S. 8,	100	o	
			Sub-group (3)		„ A 10,	1000	+++	
N 19	Serum P 1,	1500	Serum P 1,	c	[Full Titre, 1500]			
	„ C.S. 8,	100	„ C.S. 8,	tr.	Main Group I,	1000	c [A 17 = + + +]	
			Main Group I		C.S. 8,	100	o	

TABLE XIV *continued.*

Strain	Agglutination with Group I Sera		Absorption of Group I Agglutinin and consequent sub-grouping		Agglutinogenic Properties, <i>i.e.</i> Reactions of Serum prepared with strain	
					Agglutination of Spinal Strains	Absorption with same Spinal Strains
N 29	Serum P 1,	500	Serum P 1,	tr.	[Full Titre, 1000]	
	„ C.S. 8,	500	„ C.S. 8,	o	Main Group I,	100
	„ A 13,	1500	„ A 13,	c	Group C.S. 8,	o
			Sub-group (2)		„ A 13, A 10,	1000
N 48	Serum P 1,	100	Serum P 1,	+	[Full Titre, 1000]	
					Main Group I,	100
			? Sub-group (5)		Group A 13,	500
					Unidentified	
					B 3, B 10, A 1,	1000
N 58	Serum P 1,	100	Serum P 1,	o	A 16,	
	„ A 2,	500	„ A 2,	+++	Group II,	100
N 67	Serum P 1,	100	Serum P 1,	o		
	„ A 2,	500	„ A 2,	+++		

In the case of one strain, N 18, not included in the table, the evidence of relationship to Group I is confined to the single fact that it agglutinates much better with Group I than with Group II sera. Absorption tests, however, fail to identify it with any spinal strain, and its agglutinogenic action has not been ascertained owing to its extreme toxicity for rabbits.

There remain fifteen naso-pharyngeal strains which resemble the spinal strains from cases in children, B 1, B 5, B 3, and B 10, in their poor reaction with all the spinal sera tested.

Eleven of these, N 12, N 14, N 16, N 20, N 26, N 37, N 49, N 64, N 65, N 66, and N 68, agglutinate completely up to 1-500 with serum P 2, but absorb insignificant amounts of P 2 agglutinin (*vide* Table XIII). They show at most traces of agglutination with the various Group I sera, and, like the spinal strains referred to above, cannot be definitely brought into relationship with each other or with any of the other spinal strains. Their nearest allies, to judge by agglutination, are the Group II strains; perhaps sera prepared with the more divergent members of this group might identify these unclassified spinal and naso-pharyngeal strains, but in default of such sera they must all be placed as *incertae sedis*.

Of the remaining four strains, N 53, N 30, N 23 and N 24, strain N 53, the strain isolated from a soldier who had been pronounced to be a "chronic carrier," similarly unidentified by absorption, agglutinates

completely at 1-100 but not higher with sera P 2 and A 2, fails to absorb from both these sera, and neither agglutinates nor absorbs agglutinin with the others: its position in the meningococcus category is thus still more indefinite. *Strain N 30*, however, though agglutinating feebly or not at all with the spinal sera, is strongly agglutinated by the serum prepared with N 1, and absorbs almost all its agglutinin: but N 1 has been shown to conform to the tests of specific identity with the spinal strain C.S. 8, so that strain N 30, though itself divergent from this spinal strain, evidently possesses a protein-molecule resembling to a large extent that of the strain N 1; and this molecule, as has been seen, can carry combining properties of high valency for the C.S. 8 group serum: N 30, therefore, is linked up to the sub-group (1) through the intermediary strain N 1. Similarly, *strains N 23 and N 24*, though agglutinating relatively feebly with spinal sera, agglutinate up to 1-800 with the serum prepared with strain N 48, and absorb most of its agglutinin. This N 48 serum, as has been noted, agglutinates to full titre, and is absorbed by, certain strains of spinal origin which themselves were unidentified by the use of spinal sera, and were hence put in the "scrap-heaps," sub-group (5) or sub-group (7).

Had I prepared sera with such spinal strains they might have resembled serum A 2 in the peculiar restriction of their specific properties, and, like it, might have picked out and identified with themselves naso-pharyngeal strains such as N 23 and N 24, which, in absence of the appropriate spinal serum, can be brought into relationship with strains of known pathogenicity only in virtue of their relationship to another naso-pharyngeal strain N 48.

RELATIVE PROPORTIONS OF THE DIFFERENT GROUPS.

(a) *In cerebro-spinal and naso-pharyngeal strains.*

In my collection of the former, 55 per cent. were allotted to Group I and of these almost three-fourths were of one type as determined by absorption tests, and this was therefore called the main Group I. Among the naso-pharyngeal strains only 22 per cent. were related to Group I, and of these only 6 per cent. belonged to the main group of this, while 25 per cent. belonged to the rare sub-group (4), of which A 2 is the type: the other sub-groups (2), (3) and (5) having, as among the spinal strains, one or two members each.

On the other hand (as I have already mentioned), the main Group II included 56 per cent. of the naso-pharyngeal strains and only 35 per

cent. of those of spinal origin, while 8.5 per cent. of the spinal strains and 21 per cent. of the naso-pharyngeal remain ungrouped.

It has been suggested in discussing spinal strains (p. 228) that the difference in relative proportions of the different groups may indicate that the serological qualities of the different members may not depend on fixed characters, but are subject to modification as the result of their environment. It might seem from the above statistics that the Group II condition of the meningococcus was more characteristic of it while sojourning in the naso-pharynx, and that in that region modification in the direction of Group I rarely reached the full degree as represented by the main group of the Group I spinal strains, but that it halted at the stage represented by the sub-groups of Group I, which are less distantly related to Group II. It is equally possible that residence in the naso-pharynx tends to modify Group I strains in the direction of Group II.

But the most important indication furnished by a comparison between the groups found in the two classes is that each serological type found among the strains of pathogenic origin is represented among those from the normal naso-pharynx, and it seems probable that, if a sufficient number of pathogenic strains were examined for comparison, all the naso-pharyngeal strains would find an identical type among these.

In 58 of my 71 naso-pharyngeal strains sufficient serological similarity exists to permit of identification with strains of cerebro-spinal origin by complete or well-marked absorption of the agglutinin fitting these, while only 13 remain in which relationship is suggested by agglutination but has not been confirmed by absorption. The complete identification of these 13 strains might involve prolonged search for spinal strains similarly aberrant and the production of many sera with these before the exact type of aberration in each naso-pharyngeal strain met its prototype among those of spinal origin. It is evident, I think, that, given the necessary time and material, for any type of coccus in the naso-pharynx possessing the morphological and cultural characters of the meningococcus a counterpart may be found, possessing its more or less peculiar serological reactions, among cultures from the meninges.

(b) As regards age of patient and severity of disease produced.

In 36 of my 60 spinal strains I know the age of the patient from whom the strain was cultivated. Of these, 25 were of 13 years or under, while 12 were over 13. Of the 25 strains from children, 9 were classed as

Group I and 16 as Group II, while of the 12 adult strains 9 were of Group I and 3 of Group II. Though these are small figures from which to draw deductions, there is some indication that Group II affects children more than adults.

Twenty cases recovered and 8 died, the fate of the others being unknown to me. Of the cases which recovered 14 belonged to Group I and 6 to Group II, while 2 Group I cases died and 6 Group II. One cannot argue from this that Group II strains produce the severer infections; my results are probably due to their greater incidence on infants. This greater incidence of Group II on infants may indicate that the infection of children is frequently acquired from normal adults who, as has been shown, are apt to harbour Group II strains more than Group I.

SCIENTIFIC AND PRACTICAL VALUE OF SEROLOGICAL TESTS.

(a) *Absorption.*

I have now described more or less fully the serological reactions of 131 strains, of which 60 were of known pathogenicity, while 71 had shown no evidence of capacity for producing disease in the particular host.

In the first place, the value of these reactions in differentiating groups within the broad meningococcus category requires consideration. It has been seen that on the strength of agglutination two main groups are well defined: members of each group agglutinate strongly with the sera prepared by inoculation of members of the same group but feebly or not at all with sera prepared from members of the other group.

But on subjecting these main groups to more elaborate serological tests differences appear among strains which on simple agglutination would at first sight be grouped together. These differences already begin to reveal themselves when careful estimation is made of relative agglutinability with different sera and are still more marked when the power of absorbing agglutinin is taken into account. In many cases, indeed, these differences in absorptive character are absolute, *i.e.* there is either complete absorption, indicating perfect adaptation of the bacterial receptors towards the agglutinin, or complete absence of absorption, showing that the special combining parts of the protein molecule for the particular agglutinin are entirely lacking.

By some authorities¹, as I mentioned before, such differentiation is regarded as sufficient for the erection of species among strains belonging

¹ Bainbridge and O'Brien (1911), *Journ. of Hygiene*, xi. 68.

to the Food-Poisoning Group which are otherwise indistinguishable from each other. There some support is given to such an attitude by the differences in pathogenic action said to be shown by the proposed species, the one being associated with continued fever and the other with acute enteritis. But in the case of the meningococcus there is little evidence of difference in the disease produced by the different groups, and the question arises, What is the significance of the limitation to certain strains of the power of absorbing agglutinins? Is one justified in saying that the capacity for absorbing each other's agglutinin, which is found to be the common property of certain strains but absent from others, is sufficient to define the former as fixed types or species?

The final answer to such questions cannot, of course, be given until bacteriologists agree as to the criteria necessary for the erection of species among bacteria. But if it is agreed that this character, the absorption of each other's agglutinin, defines strains as belonging to a fixed type or species, then the number of such fixed types or species of meningococcus must be taken as very large, since, even in the small collection of strains which I have been able to study, there appear on this basis at least eight of them. It follows that in the placing of an unknown strain in relation to its proper type on this principle of classification at least eight monovalent sera must be applied. Yet even eight sera would fail to classify by absorption all the meningococci found producing disease.

Further, although in many cases the special power of absorbing a certain agglutinin appears sharply and definitely peculiar to certain strains, in others it appears to depend on quantitative rather than on qualitative differences, and the presence or absence of absorptive capacity is much less sharply defined. For example, in the case of strains C.S. 4, A 13, A 23, and A 10 absorption tests might lead to each being given the status of a separate type or to all being put together according as large or small quantities of culture were employed in the reaction.

Added to this difficulty, there is a serious element of confusion arising from the fact, already noted, that the same strain may vary in absorptive capacity on different occasions. Even with the main groups doubt may arise as to the type to which a particular strain should be assigned: for example, the Group II strain C.S. 16 has been seen to absorb agglutinin completely, not only from all the typical Group II sera, but also from the Group I serum A 10.

The conclusion, I think, should be that peculiarities in the absorption of agglutinin are not of sufficient permanence and are not sufficiently sharply defined in all cases to permit of their employment in the creation of hard-and-fast types or species. If they are investigated and determined with all the accuracy possible, it is evident from the number of distinct sub-groups which I have demonstrated in my small collection of strains that a useless multiplicity of types would be created; if rougher tests are depended on, the different types shade into each other and no boundary line can be drawn.

But in the meantime the chief interest of these elaborate serological reactions is the practical one. Can one by their use distinguish a meningococcus of pathogenic origin from other cocci morphologically and culturally identical?

There can be no doubt that the positive demonstration of mutual absorptive capacity for each other's sera proves sufficiently the serological identity of two strains; and such proof involves the admission that where one of the two is known to have caused disease, the other, whatever its origin, must be capable, under the proper conditions, of doing the same. Even when absorptive capacity has been demonstrated only for one strain with the serum of the other, *i.e.* where a nasopharyngeal strain absorbs the agglutinin from a spinal serum, it is probable that the same proof and admission may be allowed.

But it does not at all follow that the failure to demonstrate such identity by absorption tests excludes the coccus of unknown pathogenicity from the category of possible pathogenic strains. For it is evident, even in my small collection, that differences sufficient to prevent identification with each other are already numerous among the known pathogenic meningococci, and the probability of success in such identification is in direct proportion to the number of different sera used.

To take a concrete example, suppose the known pathogenic strains A 7 and C.S. 20 had been submitted for diagnosis as being of doubtful pathogenicity, and suppose, as might easily have happened since the group to which it belongs forms only 5 per cent. of my total, that the serum C.S. 8 had not been prepared or used, then these two strains would have been tested in vain against at least four different sera; if the positive result of an absorption test had been regarded as essential, they would have remained as doubtful meningococci or might even have been excluded altogether.

Hence the practical attitude towards the absorption test for the identification of unknown strains with pathogenic meningococci should

be that a positive outcome is decisive, that a negative result means nothing, and that even when this latter repeats itself with several different sera it does not exclude the strain from possessing pathogenicity.

(b) *Agglutination.*

Although the absorption test is too precise in its action and might exclude not only strains from the naso-pharynx of indeterminate serological character, but also known pathogenic strains, it remains for discussion whether simple agglutination might not furnish a criterion distinguishing naso-pharyngeal strains of common occurrence among non-contacts from those of spinal origin. Simple agglutination is less precise in its action and not confined as in the case of the absorption test to strains exactly identical with the strain producing the serum. Some authorities maintain that by simple agglutination a rough line could be drawn including the great majority of the pathogenic strains and excluding a large proportion of the strains commonly occurring in the normal naso-pharynx, and that it constitutes, therefore, a most useful criterion for practical use in determining the danger to be attached to meningococcus carriers.

This is the view taken by Colonel Mervyn Gordon in his recommendations for the control of cerebro-spinal fever in the Army¹, his reasoning, I think, being that naso-pharyngeal strains which fail to reach the full agglutinin titre with any of his four types of serum prepared from pathogenic strains are to be distinguished from strains of epidemiological significance.

My results indicate that there are many more than four agglutination types among pathogenic meningococci, since I have found at least eight and even then have left some unidentified.

It might theoretically be possible, however, to collect and classify a representative series of pathogenic strains and to prepare with them a manageable set of sera, to one of which, at least, any meningococcus of cerebro-spinal origin would respond in positive fashion: and it would make the task easier if abnormal strains occurring in sporadic cases and among infants could be ignored. In such circumstances a coccus from the naso-pharynx which failed to agglutinate satisfactorily might be put down either as not pathogenic or as belonging to a type producing disease so rarely that it escaped collection, and hence must be of no epidemiological importance.

¹ *Medical Research Committee, Special Report Series, No. 3, p. 7.*

But against this is the difficulty that variations in agglutinability, as has been shown, are very great and might easily lead to the exclusion of pathogenic strains if one test only were applied; indeed, with the naso-pharyngeal strains in particular, agglutinability is often much less pronounced in the first sub-culture than in succeeding ones.

Still more important as affecting the use of naso-pharyngeal swabbing in the control of cerebro-spinal fever is the prevalence in the normal naso-pharynx of meningococci of typical serological character. This is so great that the most elaborate investigation of naso-pharyngeal strains could only exclude quite a minor proportion of the total found; the great majority are identical with types of pathogenic strains of frequent occurrence in the epidemic disease and exclusion of the few which remain unidentified with my pathogenic strains would not modify the epidemiological problem represented by the large percentage of positive findings which I have demonstrated among normal persons.

PREVALENCE OF NASO-PHARYNGEAL MENINGOCOCCI IN THE VARIOUS GROUPS OF PERSONS EXAMINED.

In my first report on this subject I showed that among the out-patients attending Lambeth Infirmary in June and July, 1915, 22 per cent., 30 out of 138 examined, although they had had no relation to cerebro-spinal fever, harboured in their naso-pharynx micro-organisms indistinguishable from the meningococcus in morphology and culture, while in 13·7 per cent., 19 out of the total, the serological reactions of the strains isolated confirmed their meningococcal nature. During May, 1915, 56 school children attending a rural school in Kent were similarly examined with negative results, one strain only being found which on cultural and morphological characters resembled the meningococcus; it failed, however, to respond to the serological tests applied.

In the present report additional material in the form of naso-pharyngeal strains has been collected, and these as well as the strains previously described have been submitted to more elaborate serological examination.

On January 20, 1916, 20 children, infants under seven years attending an urban school, were examined. Again only one suspicious strain was found; it failed to agglutinate with any serum, and died out before further tests could be performed.

During the four months February to May, 1916, I had, in the course of my public health duties in a small area in North-East Kent, an opportunity of investigating the carrier percentage among soldiers and of

comparing the results found where cerebro-spinal fever had occurred with those among men who had had no connection with the disease. Omitting the school children, I have thus a set of strains from civilians (Lambeth) dating from 1915, and seven sets of soldiers, a total of 142, dating from 1916, each set forming a sample of a different body of men but in each case of men living in the conditions of close association found in camp or barracks.

The general result has been to confirm the conclusion arrived at in my first report that meningococci are to be found in a considerable percentage of persons in whom no relation to cases of cerebro-spinal fever is discoverable. In the case of the Lambeth population the percentage, as revised in the light of more elaborate study of the strains, and on the basis of full serological identification, is 11·6 per cent., while among the soldiers the percentage on the same basis varied from 10·5 per cent. to 57·6 per cent. among those in whom relation to the disease was either absent or remote, and from 25·9 per cent. to 37·5 per cent. among those in direct contact with cases.

In the following table (Table XV) the various points of importance in connection with each set are given for comparison, together with the percentages of persons in whom meningococci were cultivated from the naso-pharynx.

In analysing this table it will be best to discuss first each set *seriatim* and to reserve the general considerations to the end.

In the first set, the Lambeth out-patients, out of 138 individuals, 30, or 22 per cent., furnished strains culturally identical with the meningococcus, 19 of which, making 13·7 per cent. of the total, were also similar serologically.

Of these 30 strains 16 have been submitted to more elaborate serological tests, the rest having accidentally perished in the interval.

The following are the results of this reinvestigation:

In First Report	On more elaborate tests	
	Complete identity	Incomplete proof of identity
Identified by agglutination		
11 strains	8 strains	3 strains
Identified by culture		
5 strains	1 strain	4 strains
16	9	7

As these 16 strains have not been selected in any way, the remainder having been eliminated by accident during sub-culture, they may be considered as fairly representing the original 30; calculated on this

TABLE XV.

Meningococcus carriers in the different population groups.

No. of group	Description of group and relation to whole population	Date examined	Relation to cerebro-spinal fever before examination	No. examined	Percentage with meningococci identified completely by absorption tests per cent.	Percentage including meningococci which show relationship by absorption tests but not identity per cent.	Percentage including meningococci agglutinating with anti-meningococcus sera but not absorbing per cent.	Relation to cerebro-spinal fever after examination
1	Lambeth Out-Patients (revised statistics)	June 1-July 15, 1915	None demonstrable	138	11.6	16	22	None known
2	Soldiers, Garrison A of 500 men	Feb. 17, 1916	None	20	20	25	35	None
3	Soldiers in huts, Battalion at M. of 2000	March 2, 1916	None	19	10.5	15.5	21	2 cases in Battalion 3 months later
4	R.A.M.C. staff of Battalion at T.	March 23, 1916	2 cases in connection with Battalion 1 week before, not direct	5	40	60	60	None
5	Soldiers of Battalion at T. of 1500 men	March 28, 1916	The above 2 cases, but not direct	19	57.6	57.6	63.2	None
6	Six soldiers, 2 civilians in billets in Town S.	March 20 (4)-April 5 (4), 1916	Direct contacts of cases	8	37.5	37.5	37.5	None
7	Soldiers in huts of Battalion at M. as in (3)	May 11, 1916	None	18	33.3	33.3	38.9	2 cases, not in same huts, but in Battalion on May 24
8	Soldiers in huts of Battalion at M. as in (3) and (7)	May 26, 1916	2 cases in same huts, direct contacts	54	25.9	25.9	33.3	None
TOTALS.								
Civil	Group 1	1915	Non-contact	138	11.6	16	22	
Military	Groups 2, 3, 7	1916	Non-contact	57	21	24.5	31.5	
	Groups 4, 5	1916	Doubtful indirect contacts	24	54	58	62.5	
	Groups 6, 8	1916	Direct contacts	62	27	27	34	

proportion the percentages of persons harbouring meningococci of the same serological types as those found in cerebro-spinal fever is reduced to 11·6 per cent., as shown above. None of the 138 persons examined had had any connection with cases of meningitis as far as careful inquiry could determine, and no case of cerebro-spinal fever has occurred to my knowledge among them since.

The next group, No. 2, dates from February 17, 1916, about nine months later, and consists of 20 soldiers collected in four batches of five from different rooms in garrison barracks. The total strength of the garrison at the time was about 500 men; the sample taken, though small, was, I think, fairly representative. There had been no case of cerebro-spinal fever in the garrison for at least two years.

Four of these men, one out of each batch of five, gave cultures of typical meningococci, serologically identical with types apparently common in the epidemic disease; the percentage is thus 20 per cent. If the strain is included which showed serological relationship but not identity, the percentage rises to 25 per cent., while if all cases are included in which strains culturally identical were found the percentage becomes 35 per cent.

The next group in chronological order, Group 3, consisted of 19 men (20 were swabbed, but from one man both plates were overgrown with contaminating organisms, so that he is excluded from the set). These were swabbed on March 2, 1916, and came from soldiers of four different training companies living in huts in and near a coast village in Kent. Some 2000 soldiers in all were encamped there, and no case of cerebro-spinal fever had occurred among them since the construction of the camp. Two of these men were found to be carrying typical meningococci; from two others other two strains were cultivated, one of which showed definite relationship to pathogenic strains by absorption, while the other could not be identified thus although it agglutinated to some extent with specific sera. The percentage of positive carriers on the first basis is thus 10·5 per cent., on the second 15·5 per cent., and on the third 21 per cent.

The next group, Group 4, was examined on March 23, and consisted of five men on duty in the medical inspection room attached to a training battalion of about 1500 men in camp in a rural parish in Kent, but about a mile from a small town.

Two cases of cerebro-spinal fever had occurred in connection with this battalion one week before; one case was a soldier belonging to it who developed the disease and died while away on leave; the other was

an infant living in a small house where two of the soldiers were billeted. Indirect connection probably occurred between the contacts of these cases and the medical staff, though none of an intimate character, as far as is known.

Two of the five were found to be carrying typical meningococci, while a third yielded a strain showing definite relationship by absorption tests; the percentage of carriers was thus 40 per cent. on the first basis and 60 per cent. on the second.

Group 5 consists of 19 men of the battalion just described. They came up for vaccination, being recent recruits, on March 28, 1916, and the opportunity was taken to examine their naso-pharynx. They had been living for a fortnight under canvas, but not all in the same tent. Their relation to cases of cerebro-spinal fever is similar to that described in connection with the previous group but more remote. Eleven of the 19 men were found to be carrying typical meningococci, and a twelfth yielded a strain related by agglutination reactions but failing to absorb. On the first basis 57 per cent. were carriers, on the second 63 per cent. A month later I examined the carriers again and found in every case meningococci of the same typical character.

Groups 6, 7 and 8 were all connected with cases of cerebro-spinal fever either immediately before or after swabbing, as contrasted with those hitherto described in whom connection with this disease was either absent or remote. In Groups 6 and 8 swabbing was done after direct connection with a case had been established; in Group 7 two cases occurred a fortnight after though not in direct connection.

Group 6 consists of contacts in billets of two cases in a small town in Kent, both soldiers, but of different battalions, the contacts consisting of three soldiers and one civilian in each case. The first case occurred on March 29, the second on April 5, the contacts of each being examined the following day. Two of the contacts with one case and one with the other were found to be carrying typical meningococci indistinguishable by absorption tests from pathogenic strains and from the 11 strains found in the previous group. The percentage on the total of eight contacts is thus 37·5 per cent. No restrictive measures were adopted in the case of one, a civilian; the soldiers were isolated by the military authorities concerned. No other cases appeared in the town during 1916.

Group 7 is drawn from the same battalion as Group 3, but consists of 18 different men living under the same conditions in the same place and collected from eight different companies. They were swabbed on May 11, ten weeks after the first batch described under Group 3. No

cerebro-spinal fever had occurred in the interval. Six of the 18, or 33 per cent. yielded typical meningococci, and one other gave an atypical strain which agglutinated up to 1-500, but failed to absorb agglutinin. If this one is included the percentage of carriers is 39 per cent.

The contacts living in the same two huts as two cases of the disease constitute Group 8, and were examined two days after the appearance of the disease. None of these men had come into the previous selections made for Groups 3 and 7. Out of the 54 contacts 14 yielded typical meningococci, a percentage of 26 per cent., while four others gave strains which agglutinated somewhat feebly with specific sera and failed to absorb agglutinin. If these four are included, the total carriers amount to 33.3 per cent.

SUMMARY AND CONCLUSIONS.

(1) Meningococci from 60 cases of cerebro-spinal fever have been submitted to serological tests and compared with meningococcus-like micro-organisms from the naso-pharynx of 71 normal persons. In these serological tests 19 monovalent anti-meningococcus sera were employed, of which 11 were prepared with meningococci of known pathogenic origin and eight with strains isolated from the naso-pharynx. The tests comprised careful estimation of the agglutinability of these 131 strains with the 19 different sera and also of the extent to which these strains absorbed and removed the agglutinin from one or more sera.

(2) The simple agglutination reactions effected a rough sub-division of these 131 strains into two groups, Group I and Group II, and indicated that in Group I there was a main group comprising the majority and at least five smaller groups each comprising a few strains only, while in Group II again a main group appeared comprising the majority and at least two smaller groups containing a few strains only.

(3) Tests for absorption of agglutinin confirmed this rough sub-division into two groups and distinguished with much greater precision the different main groups and smaller groups. In general, each of these groups was found to differ in that they did not absorb the agglutinin for members of the others, and members of each produced sera from which members of the others similarly failed to absorb agglutinin.

(4) Two of the smaller groups, however, represent two "scrap-heaps" of strains unidentified by absorption reactions but suggesting by their agglutination reactions that they belonged to Groups I and II respectively. In the former there were placed three strains of cerebro-spinal and one strain of naso-pharyngeal origin, and in the latter four strains of the former origin and thirteen strains of the latter.

(5) The other main groups and smaller groups contained representatives of both cerebro-spinal and non-contact naso-pharyngeal strains, though not in equal numbers: in the main group of Group I there were placed 24 spinal strains and only one non-contact naso-pharyngeal; in the main group of Group II there were placed 16 spinal strains and 40 naso-pharyngeal, of which 23 were from non-contacts and 13 from contacts.

(6) Variations in agglutinability and absorptive capacity were shown to be so great as to interfere seriously with the use of serological tests for identifying meningococci in practice.

(7) An additional difficulty affecting both the identification of meningococci and their classification into types or groups and sub-groups is that, even with seven sera, each corresponding to a different group, strains were found, both spinal and naso-pharyngeal, which failed to react typically with any and therefore could neither be ascribed to a particular type or group nor be identified on serological grounds with the other pathogenic strains.

(8) Hence it was concluded (*a*) that it is impossible to regard these types or groups as representing distinct classes limited by hard and fast lines, and (*b*) that it is unsafe to exclude any strain from possible pathogenicity on the ground of its failure to agree serologically with any of such sets of sera as are likely to be available in practice, since even the large series I employed failed to include all pathogenic strains.

(9) The conclusion in my first report is therefore maintained that any strain possessing the admitted morphological and cultural characters of the meningococcus should be regarded as potentially pathogenic without considering its serological reactions.

(10) I have found, however, that the strains obtained from the majority of carriers show by absorption tests complete serological identity with known pathogenic strains. To this criterion 58 of my 71 naso-pharyngeal strains conform. These were distributed as follows: 16 out of 138 out-patients attending Lambeth Infirmary in June and July, 1915 (11·6 per cent.); 12 out of 57 non-contact soldiers in February, March and May, 1916 (21 per cent.); 13 out of 24 soldiers in March, 1916, who had had no direct connection with cerebro-spinal fever but in whose neighbourhood two cases had occurred (54 per cent.); 17 out of 62 soldiers who had been in direct contact with cases of the disease (27 per cent.).

(11) Inclusion of those persons who were found to be carrying strains not fully identified serologically did not raise the percentage of carriers to a significant extent.

A BACTERIOLOGICAL INVESTIGATION OF ORGANISMS RESEMBLING THE MENINGOCOCCUS FOUND BY EXAMINATION OF THE NASOPHARYNX OF PERSONS WHO HAD NOT BEEN IN CONTACT WITH PATIENTS SUFFERING FROM CEREBRO-SPINAL FEVER¹.

BY CONSTANT PONDER, M.D., CAPTAIN R.A.M.C. (T.).

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INTRODUCTION.

THE presence of the meningococcus, or an organism indistinguishable from it by any known tests, in the naso-pharynx of persons who have not suffered from cerebro-spinal fever, nor been in contact with other persons suffering from this disease, has been studied during the year 1915 by Drs Eastwood, Griffith and Scott¹. Their results showed that in the London District an unexpectedly large proportion of persons examined were carriers of this organism. In view of these results the Medical Officer of the Local Government Board considered it desirable that a further investigation should be made in a different district, where the incidence of cerebro-spinal fever had been low, and where, consequently, chances of spread of the meningococcus by direct contact with the disease would be small. The Eastern Counties seemed to meet these requirements, and accordingly, after the War Office had loaned my services to the Local Government Board for this purpose, I was instructed by the Board's Medical Officer to make investigation on the subject in question amongst the inhabitants of Cambridge, Norwich, and the surrounding areas.

I am greatly indebted to Drs Eastwood, Griffith and Scott for their valuable help during the investigation; by utilising their experiences fully in regard to media and methods a good deal of preliminary work was avoided.

The earlier part of my work, from June to October, was done in the Pathological Laboratories of the Medical Schools at Cambridge, for the use of which I wish to thank Professor Woodhead.

I am also much indebted for help, at Cambridge, to Dr Laird, the Medical Officer of Health of the Borough, Captain J. C. Graham, Dr Varrier Jones and Dr Carter (of Soham). At Norwich, Dr Cooper Pattin (the Medical Officer of Health), Dr Claridge, and Dr Long gave me every assistance. Dr Claridge, Pathologist to the Norfolk and Norwich Hospital, very kindly placed his laboratory at my disposal during my visit to the city.

The latter part of the investigation, from October, 1916, to February, 1917, was devoted to the serological examination of the strains found and was done in the Board's Laboratories.

¹ *Journ. of Hygiene*, xv. pp. 405-484.

INCIDENCE OF CEREBRO-SPINAL MENINGITIS AMONGST THE
POPULATION IN THE AREAS INVESTIGATED.

The following summary, which is prepared from information kindly supplied by the Medical Officers of Health of Cambridge and Norwich, gives details of all cases notified during 1916.

*Cases of Cerebro-spinal Fever notified in Cambridge and Norwich
between Jan. 1 and Dec. 31, 1916.*

	Patient	Date	Address	Termination	Notes
Cambridge (Pop. 40,027)	Girl (age 6)	Mar. 27th	Outskirts of town	Recovered	Only father and mother came in contact. No examination of throats was made
	Girl (age 13)	Mar. 20th	Outskirts of town	Died	First notified as enteric fever and removed to general hospital. No examination made
Norwich (Pop. 121,478)	Woman	Feb. 3rd	Town	Recovered	The naso-pharynx of ten contacts examined and one found positive
	Woman	Feb. 15th	Town	Died	Two contacts examined, none found positive
	Soldier	Feb. 28th	Town	Recovered	Eight contacts examined, five found positive
	Man	Mar. 11th	Town	Recovered	Ten contacts examined, none found positive
	Girl	Apl. 18th	Town	Died	Four contacts examined, none found positive
	Man	June 8th	Town	Recovered	Eight contacts examined, none found positive
	Soldier	Nov. 7th	Town	Died	Contacts swabbed, nega- tive

No cases were notified at either place whilst my work there was in progress, and only one subsequently.

Considering the large population of these places and the small number of cases of the disease which occurred before the investigation had begun, it will be agreed that the number of persons who can have come in *direct* contact with patients must have been very small. How far *indirect* contact may have occurred it is impossible to say.

Attempts were made to include amongst the persons chosen for swabbing individuals of different classes, living under different circumstances and in different states of health.

The swabbings were carried out in three batches:

(1) Two hundred swabs were taken in Cambridge during June and July, 1916, mostly from the naso-pharynx of out-patients at Addenbrooke's Hospital and their friends. Nearly all these patients were attending the eye department for slight defects and may, therefore, for the purposes of the investigation, be classed as normal people, since they were able to go about and in most cases do their work.

A few, however, taken at a Tuberculosis Clinic, came from patients suffering from phthisis in different stages, and the health of these patients must be classed as "impaired."

(2) A hundred swabs were taken at Norwich during August, 1916, from the naso-pharynx of medical and surgical out-patients and their friends attending the Norfolk and Norwich Hospital. Many of these patients had impaired health but all were well enough to be up and about.

(3) A hundred swabs were taken during October, 1916, amongst the employees at a factory in Cambridge where scientific instruments, etc., are manufactured. These employees were all in good health and, as they were all earning good wages, it may be assumed that their home circumstances were on the whole much more comfortable than those of the other two groups of persons swabbed.

METHODS ADOPTED IN THE INVESTIGATION.

Method of Swabbing.

Success in recovering such a delicate organism as the meningococcus depends, I think, a good deal on the amount of care given to the taking of the swab. A simple form of swab is efficient if properly prepared. In this investigation I used a plain aluminium or tinned-wire rod; the end around which the wool was wrapped was bent round to an angle. Experience soon showed that this angle must be carefully adjusted; if it was too obtuse, the handle-end of the swab impinged on the lower teeth and the wool-wrapped end could not pass properly behind the soft palate, since its point struck the posterior wall of the pharynx. The angle should be slightly larger than a right angle. Wool was wrapped round the bent end so that no metal was exposed, and the part protruding beyond the end was cut off with scissors; thus the swab ended in a flat padded surface to which mucus easily adhered. I preferred such a swab to the guarded kind, as one could guide the point with more accuracy; there was also a larger surface of wool brought in contact with mucous membrane.

The patient was placed in a good light and instructed to open the mouth as wide as possible. The tongue was depressed by a wooden spatula placed as far back as convenient. The swab then being held ready on the dry upper surface of the spatula, the patient was told to phonate the sound "ah," and, directly the soft palate rose as a result of this action, the bent end of the swab was passed up behind the soft palate. In carrying out this process the outer part of the wool is rubbed on the back wall and vault of the naso-pharynx, and, if carefully conducted, there is no possibility of contamination by saliva. That surface of the swab which had come in contact with the naso-pharynx was then rubbed over an ascitic-agar plate, taking care to transfer to the surface of the medium any beads or threads of mucus which had adhered to the wool.

Media and Cultivation of Swabs.

The plate to which the mucus was transferred contained ascitic-agar made from ordinary nutrient agar (reaction + 8) containing 2.5 per cent. agar. This, after being melted and cooled to 55° C., had received the addition of sterile ascitic fluid in the proportion of one part ascitic fluid to three parts nutrient agar.

The plates were taken to the laboratory without delay; but it was not found essential to keep them at blood temperature in a portable incubator, as some investigators have stated. The mucus was spread out on the plate by means of a bent glass rod, care being taken to tease it out as thoroughly as possible. The same glass rod was then wiped lightly over the surface of a second plate. The rod should only pass once over the surface, and a large plate is obviously better than a small one. This second plate contained Kutscher's medium prepared as follows: to a broth made from fresh human placenta, 500 grms. to the litre, are added nutrose 2 per cent., glucose 1 per cent., peptone (Chapoteaut) 1.5 per cent., and agar 2.5 per cent.; the reaction is brought to + 8 (Eyre's scale) after steaming. Tubes containing a convenient amount are melted, cooled to 55° C., and sterile filtered ox serum is added in the proportion of one part to three; the medium is then ready to pour into the Petri dish.

The plates were examined after 24 hours and after 48 hours; if the second plate was spread as described above, a sufficient number of colonies, not overcrowded, was obtained almost invariably. The primary ascitic-agar plate was usually overcrowded with colonies, and was only examined when the Kutscher plate contained very few colonies.

Examination of Cultures and Preservation of Strains.

After some experience the colonies of meningococcus-like organisms on Kutscher's medium can be distinguished readily, even by unaided sight, in a mixed culture such as is obtained from a throat swab. Their colour is the bluish-grey of tobacco-smoke. After 24 hours growth they are about 2 mm. in diameter, translucent, and with a lens may appear faintly granular. They are slightly raised, and the margins are regular. After 48 hours they are about 4 mm. in diameter, or even more where there is room for free growth; they preserve their original colour, translucency and faint granularity; they often show an annular appearance due to variations in thickness between the centre and circumference. In some strains the edges of the colonies may become slightly irregular and slight striation may be noticed. Colonies do not fuse readily one with another, and in the case of some strains a distinct facetting may be noticed where two or more colonies grow in contact. When emulsified, as a rule they dissolve easily like paint; some strains may be slightly glutinous and some tend to adhere slightly to the medium. This latter characteristic appears to depend on certain qualities of the media. Some strains in subculture may assume a faintly yellow tint. These slight variations were also noticed in strains of meningococcus of cerebro-spinal origin.

When the meningococcus-like organisms were present in a throat culture, they often formed a large proportion of the colonies on the plate and sometimes they were present in almost pure culture. This appears to indicate that these organisms find a suitable *habitat* in the naso-pharyngeal mucus and are well able to hold their own in competition with the other flora.

Strains retained for further examination were sown on slopes of egg medium, on which they remain alive usually for several months. This medium is prepared as follows: new-laid eggs with shells unbroken are placed one at a time in boiling water for 15 seconds, thus coagulating and sterilising the most external layer of albumen. The shells are then carefully broken on the edge of a sterile measuring cylinder, and the contents allowed to fall in without contamination. The amount is noted, and the contents of the cylinder are poured into a large sterile flask, which is shaken until all the yolks are broken. Sterile normal salt solution, in the proportion of one part to three parts of egg, is then added. The mixture is syphoned into test tubes and sterilised by inspissating in moist heat at 85° C., for two hours on each of two successive

days. The plugs of the tubes are sealed with melted paraffin to prevent the medium drying.

Identification of the Meningococcus.

Satisfactory methods for identifying the meningococcus cannot yet be said to be established. There is no practical test of pathogenicity and one is compelled to fall back on cultural and serological tests, in regard to which a definite standard of identification has not been agreed upon. Where the organism is leading a pathogenic existence, knowledge of the locality whence the strain was isolated plays a strong part in influencing the decision. For example, meningococci and gonococci have many bacteriological characteristics in common, and even serologically can only be distinguished *in vitro* by tests which are not very definite; if, however, one is cultivated from cerebro-spinal fluid and the other from a urethral discharge, there is a valuable clue for differential diagnosis. But with an organism isolated from a throat, and perhaps living a saprophytic existence there, the locality from which it is isolated gives no clue to its differentiation, since many varieties of gram-negative cocci occur in this region.

Cultural characters.

These have been described above in connection with the growth on Kutscher's medium. On subculture, certain strains were found to become yellow and glutinous; such strains often gave sugar reactions different from the meningococcus and invariably failed to agglutinate with the sera tested.

Microscopical characters.

Throat strains of meningococcus-like organisms seldom showed any difference in microscopical appearance from meningococci of cerebro-spinal origin. The presence of tetrads and larger groups of cocci varies, and seems to depend largely on conditions of growth.

Fermentation of sugars.

Strains were tested as regards their action on glucose, maltose and saccharose. The medium used was prepared according to Lingelsheim's formula. To 210 c.c. of ordinary 2.5 per cent. nutrient agar which has been melted and cooled to 55° C. are added 70 c.c. ascitic fluid, 20 c.c. of 10 per cent. solution of the required sugar, and sufficient Kubel and

Tiemann's litmus to give a good blue colour, all these latter solutions being heated to a temperature of 55° C. The medium is then syphoned into test tubes and allowed to cool in a sloped position.

On such slopes the characteristic growth of the meningococcus is readily visible, and the production of slight acidity is easily detected. A recently isolated, vigorous strain of meningococcus will usually show definite acidity on glucose and maltose slopes in 24 hours, but no acidity can be obtained on saccharose slopes. Strains that have been stored and subcultured several times may fail to produce acidity with either glucose or maltose or perhaps with both.

The sugar reactions of the great majority of the meningococcus-like organisms isolated from the throat resembled the reactions of meningococci of cerebro-spinal origin. A few were found that fermented saccharose; these did not agglutinate with the sera tested. A certain number when freshly isolated failed to give acid with either glucose or maltose; these, for the most part, were difficult to emulsify, and suspensions rapidly showed spontaneous sedimentation. All but two failed to show satisfactory agglutination with the sera used. These organisms were probably related to the type which has been described under the name *M. catarrhalis*.

Serological tests; preparation of immune sera.

Young rabbits weighing 1,500 to 2,000 grms. were inoculated intravenously at intervals of 5-7 days with increasing doses of live 24 hour ascitic-agar slope cultures. A satisfactory series of doses was found to be a half slope for each of the first two inoculations, a whole slope for each of the next two inoculations, and a slope and a half for each of the next two. In about six weeks to two months a serum which gave a titre for the homologous organism of at least 1:800 was usually obtained.

Nine monovalent sera were prepared, seven from cerebro-spinal and two from naso-pharyngeal strains.

Preparation of suspensions of cocci.

The whole of the growth from a 24 hour old ascitic-agar slope was spread by means of a glass spreader over the surface of two or three Petri plates of ascitic-agar. After 24 hours' incubation the growth was scraped off and weighed and a suspension of the required strength

was made up by adding sufficient phenol-saline solution (.5 per cent. phenol in normal saline).

For ordinary agglutination tests, the suspension was always used in a strength of 2 mg. to the c.c., making thus 1 mg. to the c.c. in the mixture of suspension and serum.

Contrary to the experience of some workers, it was found that suspensions made from growth on a medium containing ascitic fluid were not markedly less agglutinable than suspensions made from a growth on simple nutrient agar.

Conduction of agglutination tests.

The macroscopic method was always employed. The serum was diluted to strengths of 1 : 50, 1 : 100, 1 : 200 and 1 : 400; thus, when an equal quantity of coccal suspension was added, the serum was present in double this dilution, viz. 1 : 100, 1 : 200, 1 : 400, and 1 : 800. Sera were not tested in higher dilution. Half a cubic centimetre of each dilution was placed in a set of small test tubes arranged in series, and to each was added half a cubic centimetre of the suspension of the coccus to be tested. Racks holding the tubes were placed in an incubator at 55° C. for 24 hours, after which they were taken out and allowed to stand for 24 hours before reading the results.

RESULTS OF THE INVESTIGATION.

Details respecting persons found to be carrying organisms indistinguishable from the meningococcus.

The following table (Table I) gives certain details respecting the persons yielding colonies in the primary plate which resembled the meningococcus. The numbers in the left-hand column were given in sequence to each person in the order examined. In the column headed "Sugar reactions" G > M indicates that stronger acidity was produced with glucose than with maltose, while M > G indicates the reverse. G = M means equally marked acidity with the two sugars.

In the column headed "Highest agglutination" the arabic numerals indicate the highest dilution giving complete agglutination, while M I, M XIII, NP 108, etc., refer to the different monovalent sera with which this result was produced. *Vide* Table V for the agglutinating properties of these towards meningococci of cerebro-spinal origin.

TABLE I.

Details respecting persons yielding colonies in the primary plate which resembled the meningococcus.

No.	Date	Sex	Age	Physical condition	Locality	Urban or Rural	No. of colonies on original plate	Sugar reaction	Sub-cultures	Highest agglutination
7	13. vi. 16	F.	34	Phthisis	Cambridge	U.	Many	G. > M.	Like meningococcus	800 (M. i. etc.)
12	15. vi. 16	M.	29	"	Cambridgeshire	R.	Almost pure	M. > G.	"	50 (M. i.)
13	"	M.	24	Mitral disease	Cambridge	U.	Almost pure	M. = G.	"	800 (M. i. etc.)
18	17. vi. 16	F.	43	Normal	Ely	R.	Many	G. > M.	"	800 (M. i. etc.)
20	"	F.	48	"	Cambridgeshire	R.	Many	G. = M.	"	800 (M. i. etc.)
29	"	F.	45	"	"	R.	Many	G. = M.	Colonies yellow	Nil.
32	19. vi. 16	M.	53	"	Cambridge	U.	Many	G. = M.	Like meningococcus	400 (M. xiii, NP 108)
60	5. vii. 16	M.	49	Ophthalmia	"	U.	Large proportion	G. = M.	Colonies yellow	Nil.
65	"	M.	74	Normal	"	U.	Large proportion	?	Like meningococcus	800 (M. i. etc.)
68	"	F.	74	"	Ely	U.	Few	Ferments saccharose	Yellow colonies	Nil
70	"	M.	13	"	Cambridgeshire	R.	Few	G. = M.	Like meningococcus	800 (M. i. etc.)
76	7. vii. 16	M.	12	"	Cambridge	U.	Many	?	Strain lost	"
77	"	F.	41	"	"	U.	Few	M. > G.	Like meningococcus	Nil
90	10. vii. 16	M.	6	"	Cambridgeshire	R.	Many	Ferments saccharose	Yellow colonies	"
96	"	M.	6	Tonsils, etc., etc.	Cambridge	U.	"	G. = M.	Like meningococcus	"
97	"	M.	13	Normal	"	U.	"	G. = M.	Died	?
99	"	F.	7	Con genital syphilis	"	U.	"	?	Like meningococcus	800 (M. iv, etc.)
100	"	M.	4	Adenoids	"	U.	"	Ferments saccharose	Colonies yellow	Nil
101	12. vii. 16	M.	29	Phthisis (early)	Patients at Bourn	Open air life	Few	G. = M.	Like meningococcus	800 (M. iv, etc.)
106	"	M.	20	" (advanced)	enulosis colony	"	Almost pure	G. > M.	"	800 (M. iv, etc.)
108	"	M.	22	" (advanced)	Cambridgeshire	R.	Few	G. > M.	"	800 (M. i, M. iv)
109	"	M.	13	" (early)	"	"	Pure culture	M. > G.	"	200 (NP 108)
112	"	M.	17	" (early)	Cambridge	U.	Many	M. > G.	"	200 (M. xv, NP 108)
117	19. vii. 16	M.	70	Normal	"	U.	Many	M. > G.	"	800 (M. i. etc.)
121	"	F.	49	"	"	U.	Few	M. > G.	Died	?
122	"	M.	61	"	"	U.	Many	M. > G.	Like meningococcus	Nil
129	"	M.	8	Keratitis	"	U.	Almost pure	M. > G.	"	400 (M. xv, NP 108)
131	"	M.	75	Normal	Cambridgeshire	R.	One colony	M. = G.	"	"
136	21. vii. 16	F.	21	"	Cambridge	U.	Many	M. = G.	Colonies yellow	50 (M. xvi)
139	"	M.	18	"	St Ives	R.	Few	Ferments saccharose	Like meningococcus	800 (M. i. etc.)
141	"	M.	49	"	Cambridgeshire	R.	"	M. > G.	"	"

146 148	21. vii. 16 "	F. M.	78 14	Normal "	" " "	Cambridgeshire " "	R. R.	Many Few	" " "	G = M. G. = M.	" " "	" " "	Like meningococcus. Contaminated and outgrown Colonies yellow.	Nil ?
149	24. vii. 16	F.	5	"	"	Cambridge	U.	Few	"	?	"	"	Strain lost Like meningococcus. Like meningococcus.	400 (M. i. etc.) 800 (M. xiii)
150	"	M.	10	"	"	"	U.	Many Large	"	?	"	"	Colonies yellow.	Nil
153	"	F.	26	"	"	"	U.	Large proportion	"	?	"	"	Died Colonies yellow.	?
155	"	F.	38	"	"	"	U.	Large proportion	"	?	"	"	Contaminated and outgrown Like meningococcus.	800 (M. i. etc.) ?
161	29. vii. 16	F.	15	"	"	Cambridgeshire	R.	Few	"	?	"	"	Like meningococcus.	?
164	"	F.	8	"	"	"	R.	Many	"	G. = M.	"	"	Colonies yellow.	Nil
172	31. vii. 16	M.	12	"	"	Royston	U.	Many	"	G. = M.	"	"	Contaminated and outgrown Like meningococcus.	800 (M. i. etc.) ?
174	"	F.	16	"	"	Cambridge	U.	Many	"	G. = M.	"	"	Like meningococcus.	?
178	"	F.	27	"	"	"	U.	Many Large	"	G. > M.	"	"	Like meningococcus.	800 (M. xiii)
179	10. viii. 16	M.	32	"	"	"	U.	Large proportion	"	"	"	"	Like meningococcus.	?
181	"	F.	25	"	"	Cambridgeshire	R.	Few	"	?	"	"	Like meningococcus.	?
182	"	F.	36	Phthisis (early)	"	Cambridge	U.	Large proportion	"	G. > M.	"	"	Like meningococcus.	800 (M. vi. M. xiii)
183	"	M.	45	Phthisis	"	Cambridgeshire	R.	Large proportion	"	G. = M.	"	"	Like meningococcus.	?
184	"	M.	22	Normal	"	"	R.	Many	"	G. > M.	"	"	Like meningococcus.	800 (M. i. etc.)
186	"	F.	15	Phthisis (early)	"	Cambridge	U.	Few	"	M. > G.	"	"	Yellow colonies.	50 (NP 108)
188	"	F.	8	"	"	"	U.	Few	"	G. = M.	"	"	Like meningococcus.	800 (M. xvi. M. xiii NP 108)
191	"	F.	38	Normal	"	"	U.	Large proportion	"	?	"	"	Like meningococcus.	?
196	15. viii. 16	M.	69	"	"	Cambridgeshire	R.	Many	"	G. > M.	"	"	Like meningococcus.	800 (M. xiii)
198	"	M.	36	"	"	"	R.	Large proportion	"	"	"	"	Like meningococcus.	?
200	"	M.	56	"	"	"	R.	Many	"	?	"	"	Like meningococcus.	?
201	23. viii. 16	F.	10	Lupus	"	Norfolk	R.	One colony	"	G. = M.	"	"	Like meningococcus.	200 (NP 108)
202	"	F.	32	Asdima	"	Norwich	U.	Many	"	G. = M.	"	"	Like meningococcus.	800 (M. xiii)
204	"	F.	38	Anaemia	"	"	U.	Many	"	G. = M.	"	"	Like meningococcus.	50 (M. xvi. M. xiii)
207	"	F.	46	Normal	"	"	U.	Many	"	G. = M.	"	"	Like meningococcus.	200 (M. iv. NP 108, NP 235)
209	"	F.	33	Syphilis	"	"	U.	Large proportion	"	G. = M.	"	"	Like meningococcus.	?

TABLE I—continued.

Details respecting persons yielding colonies in the primary plate which resembled the *meningococcus*.

No.	Date	Sex	Age	Physical condition	Locality	Urban or Rural	No. of colonies on original plate	Sugar reaction	Sub-cultures	Highest agglutination
211	23. viii. 16	F.	25	Epilepsy	Norwich	U.	Almost pure	?	Died	?
212	"	F.	51	Goitre	"	U.	Many	G. = M.	Like meningococcus	800 (M. xvi)
214	"	F.	11	Normal	"	U.	Almost pure	G. = M.	"	800 (M. i, etc.)
216	"	F.	26	Rheum. arthritis	"	U.	Almost pure	G. = M.	Like meningococcus	800 (M. i, etc.)
217	"	M.	45	Epilepsy	"	U.	"	?	Died	?
222	26. viii. 16	M.	32	Phthisis	"	U.	Many	G. > M.	Like meningococcus	800 (NP 235)
223	"	F.	50	Normal	"	U.	Large proportion	G. = M.	"	800 (M. i, etc.)
224	"	M.	8	Chorea	"	U.	Few	M. > G.	Too adherent to medium to emulsify	?
227	"	F.	30	Normal	"	U.	"	?	Adherent to medium	?
229	"	F.	40	Normal	"	U.	Almost pure	G. = M.	Like meningococcus	800 (M. i, etc.)
232	"	F.	26	Chlorosis	"	U.	Large	G. > M.	"	800 (M. xiii, NP 235)
235	"	F.	45	Normal	"	U.	Almost pure	G. > M.	"	800 (M. i, etc.)
236	"	M.	65	Carcinoma	"	U.	Many	G. = M.	"	400 (M. xiv)
237	"	M.	30	Normal	"	U.	Almost pure	Ferments saccharose	Colonies yellow	Nil
238	"	M.	15	Septic wound	"	U.	Large proportion	?	Adherent	?
239	"	M.	15	Normal	"	U.	Many	?	Colonies yellow	?
242	"	M.	18	Normal	"	U.	Almost pure	G. = M.	Like meningococcus	800 (M. i)
244	28. viii. 16	M.	28	Deafness	"	U.	Few	Ferments saccharose	Very adherent to media	Nil
245	"	M.	19	"	Soldiers under canvas at various camps and attending the Norfolk and Norwich Hospital for ear trouble	All R.	Many	G. = M.	Like meningococcus	800 (M. xiii, NP 108, NP 235)
247	"	M.	23	Otitis	"	"	"	G. = M.	"	800 (M. i, M. xiii)
248	"	M.	21	Deafness	"	"	"	G. = M.	"	Nil
249	"	M.	24	"	"	"	"	G. = M.	Very adherent to media	50 (NP 235)
250	"	M.	30	Otitis	"	"	Almost pure	G. = M.	Like meningococcus	800 (M. i, etc.)
255	"	M.	20	Deafness	"	"	One colony	?	Died	?
256	"	M.	29	Otorrhoea	"	"	Few	G. = M.	Like meningococcus	800 (M. i, M. xiii)
257	"	M.	46	Normal	Norwich	U.	Many	G. = M.	Very sticky in sub-culture	?
259	"	F.	5	Otitis	"	U.	Few	G. = M.	Too adherent to make emulsion	"

262	28. viii. 16	M.	14	Normal	.	.	Norwich	.	U.	Large proportion	G. = M.	.	.	Like meningococcus.	800 (NP 108)
264	"	M.	18	"	.	.	"	.	U.	Few	G. = M.	.	.	Yellow colonies.	Nil
265	30. viii. 16	M.	26	Epilepsy	.	.	"	.	U.	Large proportion	G. = M.	.	.	Like meningococcus.	200 (NP 108)
267	"	F.	51	Myxoedema	.	.	"	.	U.	Few	?	?	.	Adherent	?
268	"	F.	18	Dyspepsia	.	.	"	.	U.	Large proportion	?	?	.	"	?
271	"	F.	29	Rheum. arthritis	.	.	"	.	U.	Large proportion	?	?	.	"	?
272	"	F.	36	"	.	.	"	.	U.	Few	G. = M.	.	.	"	200 (M. xv, NP 108)
276	"	F.	35	Neurasthenia	.	.	"	.	U.	"	Ferments saccharose	.	.	"	Nil
279	"	F.	15	Goitre	.	.	"	.	U.	Many	.	.	.	"	?
280	2. ix. 16	M.	39	Phthisis	.	.	"	.	U.	Few	?	.	.	"	?
281	"	M.	36	Sciatica	.	.	"	.	U.	Almost pure	G. = M.	.	.	Like meningococcus.	Nil
282	"	M.	27	Epilepsy	.	.	"	.	U.	Large proportion	G. > M.	.	.	"	800 (M. i)
283	"	M.	72	Apoplexy	.	.	"	.	U.	Almost pure	G. = M.	.	.	Like meningococcus.	100 (M. i, NP 235)
289	"	F.	25	Neurasthenia	.	.	"	.	U.	Large proportion	G. = M.	.	.	Tenacious growth would not emulsify	?
290	"	M.	48	"	.	.	"	.	U.	One colony	G. = M.	.	.	Like meningococcus.	200 (M. xiv, M. xv)
292	"	F.	46	Retrollexed uterus	.	.	Norfolk	.	R.	"	?	.	.	Died	?
295	"	M.	14	Normal	.	.	Norwich	.	U.	"	G. = M.	.	.	Like meningococcus.	200 (M. xv)
296	"	F.	14	"	.	.	"	.	U.	Almost pure	G. = M.	.	.	Colonies yellow.	800 (M. xiii)
297	"	M.	50	Carcinoma of tongue	.	.	"	.	U.	"	?	.	.	Strain lost	?
299	"	M.	9	Normal	.	.	Norfolk	.	R.	Large proportion	?	?	.	Adherent	?
305	12. x. 16	M.	31	"	.	.	Cambridge	.	U.	Three colonies	M. = G.	.	.	Like meningococcus.	800 (M. ii, M. xiii)
307	"	F.	22	"	.	.	"	.	U.	Large proportion	G. > M.	.	.	"	800 (M. i, et(c.))
310	"	F.	23	"	.	.	"	.	U.	Almost pure	G. = M.	.	.	"	800 (M. xiii)
312	"	F.	22	"	.	.	"	.	U.	Large proportion	G. > M.	.	.	"	800 (M. xiii)
315	"	M.	18	"	.	.	"	.	U.	Many	Ferments saccharose	.	.	"	100 (M. i, M. xiii, NP 108)
317	"	M.	24	"	.	.	"	.	U.	Almost pure	G. = M.	.	.	"	800 (M. xiii, NP 108, NP 235)
318	"	F.	16	"	.	.	"	.	U.	Large proportion	G. = M.	.	.	"	200 (M. i, et(c.))

TABLE I—continued.
Details respecting persons yielding colonies in the primary plate which resembled the meningococcus.

No.	Date	Sex	Age	Physical condition	Locality	Urban or Rural	No. of colonies on original plate	Sugar reaction	Sub-cultures	Highest agglutination
322	16. x. 16	M.	23	Normal	Cambridge	U.	Large proportion	G. = M.	Like meningococcus.	800 (M. i, NT 108)
333	"	M.	25	"	"	"	Many	G. = M.	"	800 (M. xiii)
334	"	M.	18	"	"	"	Few	G. = M.	"	400 (M. xiii)
335	"	M.	16	"	"	"	"	G. > M.	"	800 (M. i, etc.)
338	"	M.	18	"	"	"	Many	G. = M.	"	100 (M. i, etc.)
339	"	M.	16	"	"	"	Almost pure	G. = M.	"	400 (M. i, M. xiii)
341	"	M.	19	"	"	"	"	G. = M.	"	400 (M. xiii)
342	"	M.	29	"	"	"	Few	G. = M.	"	100 (NP 108)
345	18. x. 16	M.	60	"	"	"	Many	G. = M.	"	800 (M. i, etc.)
347	"	M.	33	"	"	"	"	G. > M.	"	800 (M. i, etc.)
348	"	M.	28	"	"	"	Almost pure	G. > M.	"	800 (M. i, etc.)
349	"	M.	17	"	"	"	"	G. = M.	"	800 (M. xiii)
350	"	M.	27	"	"	"	"	M. > G.	"	800 (M. i, M. xiii)
351	"	M.	18	"	"	"	Many	G. = M.	"	200 (M. i)
354	"	M.	17	"	"	"	"	M. > G.	"	800 (M. i, M. xiii)
356	"	M.	18	"	"	"	Large proportion	G. = M.	"	800 (M. xiii)
357	"	M.	15	"	"	"	Many	G. > M.	"	800 (M. i)
358	"	M.	20	"	"	"	Almost pure	G. = M.	"	800 (M. xiii, NP 108)
359	"	M.	23	"	"	"	Large proportion	M. > G.	"	200 (M. i, NP 235)
361	"	M.	28	"	"	"	"	G. = M.	"	800 (M. i, etc.)
362	"	M.	21	"	"	"	"	G. > M.	"	800 (M. i, etc.)
366	23. x. 16	M.	30	"	"	"	Few	G. = M.	"	?
369	"	M.	14	"	"	"	"	G. = M.	"	?
371	"	M.	19	"	"	"	Almost pure	G. = M.	"	400 (M. i)
372	"	M.	21	"	"	"	One colony	G. > M.	"	800 (M. i)
375	"	M.	15	"	"	"	Large proportion	G. = M.	"	200 (M. i, NP 108)
384	25. x. 16	M.	33	"	"	"	Few	G. = M.	"	100 (M. i, etc.)
386	"	M.	23	"	"	"	Many	G. = M.	"	800 (M. i, etc.)
387	"	M.	29	"	"	"	Few	G. = M.	"	800 (M. i, etc.)
388	"	M.	19	"	"	"	"	G. = M.	"	800 (M. i, etc.)
391	"	M.	29	"	"	"	Almost pure	G. > M.	"	800 (M. i)
396	"	M.	26	"	"	"	"	G. = M.	"	800 (M. xiii)
397	"	M.	20	"	"	"	Many	G. = M.	"	?
400	"	M.	21	"	"	"	Large proportion	G. = M.	Colonies yellow: not tested further Like meningococcus.	800 (M. i)

TABLE II.

Showing results obtained with each batch of swabs taken.

Date when collected	Where collected	Class of individual examined	Number of strains resembling meningococcus in primary plate	Strains shown to differ by cultivation, etc.	Strains died	Strains examined for agglutinability
<i>1st hundred:</i>						
June 13th— July 10th, 1916	Cambridge, Adden- brooke's Hospital and Cambridge County Tubercu- losis Clinic	Eye out- patients and their friends. Tuberculosis patients and their friends	18	6	2	10
<i>2nd hundred:</i>						
July 12th— Aug. 15th, 1916	The same: also 16 swabs at tubercu- losis colony	The same	35	5	10	20
<i>3rd hundred:</i>						
Aug. 23rd— Sept. 2nd, 1916	Norwich: Norfolk and Norwich Hos- pital	Medical and surgical out- patients and their friends	56	16	8	27
<i>4th hundred:</i>						
Oct. 12th— Oct. 25th, 1916	Cambridge: Factory X.	Employees in normal health	41	3	1	37

The proportion of positive results obtained, as shown in Table II, increased very considerably during the course of the investigation: this increase may be partly due to greater proficiency in taking swabs and to greater experience in detecting suspicious colonies. In the third batch, taken at Norwich, over 50 per cent. showed suspicious organisms in the primary plates; this number was considerably reduced on further examination, since many of the strains showed definite evidence of belonging to the *catarrhalis* type of organism rather than the meningococcus.

The analysis of results found in Table III is an attempt to show the influence of age, sex, health and surroundings on the proportion of meningococcus-like organisms found. The highest proportion seems to occur in young adult life, but it is also high in old people.

Amongst children a larger proportion of suspect organisms was obtained, but many of these could be differentiated from the meningococcus by cultivation and sugar tests, so that the number of positives in children was rather lower than in adults.

At every age suspicious colonies appear to be more commonly met with in males than in females.

There is some indication that persons with impaired health carry meningococcus-like organisms more frequently than those in good health, and town dwellers more than country dwellers. But the figures suggesting this cannot be insisted upon, as the batches were taken at different times of the year and in different localities.

The general impression is obtained that a fairly high proportion of people of both sexes and all classes and ages can be shown to carry the organisms in question.

TABLE III.

Analyses of sex, age, surroundings and physical health of 111 persons, from whose naso-pharynges were obtained organisms resembling meningococci in culture and sugar reactions.

Age Period	AGE AND SEX.									
	-15		16-35		36-60		61-		All ages	
	Total examined	Positive	Total examined	Positive	Total examined	Positive	Total examined	Positive	Total examined	Positive
Male	43	12 (28 %)	122	49 (40 %)	39	10 (26 %)	14	6 (42 %)	218	77 (35 %)
Female	29	5 (17 %)	71	14 (20 %)	71	13 (18 %)	11	2 (18 %)	182	34 (19 %)
Totals	72	17 (24 %)	193	63 (33 %)	110	23 (21 %)	25	8 (32 %)	400	111 (28 %)

HEALTH AND SURROUNDINGS.

	Total examined	Positive		Total examined	Positive
Town dwellers	267	85 (32 %)	Normal health	292	76 (26 %)
Country dwellers	133	26 (19 %)	Health impaired	108	35 (32 %)

NOTE. A few of the strains recorded in the above analyses were not fully tested owing to their having died (see Table I).

Certain interesting features may be recorded. The patients at Bourn Tuberculosis Colony were living a completely open-air life, and had been practically isolated from the general population for periods varying from a few weeks to six months. Out of 14 examined, five showed that organisms culturally and serologically resembling the meningococcus could be recovered from their naso-pharynges. From one of the strains occurring in a patient who had been in the sanatorium six months, a serum was prepared which agglutinated well both meningococci of cerebro-spinal origin and other naso-pharyngeal strains.

A number of soldiers, all on service at camps in the country districts around Norwich, attended the hospital for deafness, ear discharge, etc., otherwise they were in good health. Out of 13 examined, eight gave colonies like the meningococcus; of these strains one differed in fermenting saccharose, one was lost without being tested, one resembled *M. catarrhalis* and four gave serological reactions similar to those given by the meningococci isolated from cases of the specific disease.

Amongst the employees at the factory, an interesting point came to light which may be of importance.

The factory contains three workshops fitted with lathes and other machinery. Two of the shops, which may be called A and B, have been built and been in use some years; the ventilation in these two shops is dependent mainly on the opening of windows at the will of the men working there; the cubic space per man, while quite in accordance with regulations, is not excessive. The third workshop, which may be called C, has been built within the last year; the machinery is new, and occupies a much smaller proportion of the cubic space than in workshops A and B; the shop is also much loftier than the earlier ones. Ventilation is effected not only by open windows but also by extracting fans and other modern devices, so that conditions in this respect may be said to be as perfect as possible. The cubic space per man is very large compared with workshops A and B. In the following table a comparison between the results found amongst the employees working in these workshops is shown. There are also added the results found amongst employees in the accounts department, the drawing office and other rooms where work demanding special technical skill is carried out. It may be stated that, on the whole, the employees in these last departments receive higher salaries, and

TABLE IV.

A comparison between results obtained amongst employees working under different conditions.

	Ventilation, etc.	Cubic space per man	Number examined	Number found positive
Shop A . .	Windows: old building	682	39	24 (61%)
Shop B. .	Windows: old building	642	11	6 (55%)
Shop C. .	Fans, etc., windows: quite new building	2249	17	4 (23%)
Accounts Dept., drawing office, etc.	Various: all quite satisfactory: (5 of the employees were women)	?	15	1 (6%)

consequently live in more comfortable and probably more hygienic home circumstances. It should be noted that five of the employees in these departments were women, whereas the employees in shops A, B and C were all men.

It will be noted that a far higher proportion of positive results was obtained amongst employees working in the older, less ventilated workshops, where also cubic space was more restricted.

In view of the smallness of the figures it would be unwise to draw a too definite conclusion, but the results are suggestive.

Agglutination tests on strains collected.

Nine monovalent sera were prepared in the manner described above. These sera were utilised for testing the agglutinability of all the cocci obtained during the investigation, and also were tested against certain undoubted meningococci isolated from the cerebro-spinal fluid of patients suffering from cerebro-spinal fever. These latter strains, which were given me by Dr Scott, were chosen somewhat at random but included not only strains serologically like those predominating in the recent epidemics but also strains difficult to identify with either of the two main groups of meningococci.

The sera were prepared from the following strains:

M I. A typical example of the serological group of meningococci, which has perhaps been found most commonly in recent epidemics. The titre of the serum prepared was 800. The strain used was obtained from Dr Scott, who has designated it C.S. 16 and has found it typical of Group II.

M IV. An example of the same group, shown by absorption tests to be practically identical with *M I*. The titre of this serum was about 600.

M VI. An example of the same group. The serum did not give either with the homologous organism or with other members of the same group a sufficiently high titre, *i.e.* it did not give complete agglutination at dilutions above 1-200.

M XVI. The serum from this organism was prepared by Dr Griffith and used to test the strains I collected because it was a typical example of another common group of meningococcus. The titre of this serum was about 1000. Dr Griffith calls this strain *M 43*, and regards it as typical of Group I.

M XVII. This coccus was apparently closely allied to *M XVI*; the titre was about 800.

M XV. This coccus was allied to *M XVI* but showed definite differences when tested by absorption methods.

M XIII. This coccus was allied to the group of which *M XVI* is an example, but was not identical with it. The serum gave very variable results with suspensions prepared at different times and of different age.

NP 108. Unlike the above, which were all prepared from cerebro-spinal fluid strains, this coccus was obtained from the naso-pharynx of a man of 22 who had been isolated in a tuberculosis open-air colony for six months, suffering from advanced phthisis. The coccus resembled the meningococcus very closely, and gave similar agglutination tests to the strains *M I* and *M IV*. The titre of the serum was about 400.

NP 235. This was also a naso-pharyngeal coccus, and was obtained from a woman of 45 years of age in good health, living in Norwich. This coccus also resembled *M I* and *M IV* in its agglutinability. The titre of the serum was about 800.

In Table V are grouped the cross-agglutination tests between the test sera and certain meningococci of cerebro-spinal origin, amongst which are the cocci homologous to the sera.

Clear division into groups is not well established in this series. *M I* and *M IV* appear to belong to one group which gives consistent results with all the sera. *M XV* and *M XVI* also give evidence of being closely related to each other and of a different type from *M I* and *M IV*. But the intermediate strains give irregular results which make any classification impossible. The action upon these meningococcal strains of the sera prepared from naso-pharyngeal strains indicates that the latter were more nearly allied to *M I* and *M IV* than to *M XVI*, but hardly gives a basis for definite grouping.

The testing of a large number of strains by agglutination tests presents many technical difficulties. Chief amongst them is the variability of the coccus. Unless the sera are all prepared ready in anticipation, it is impossible to examine the cocci when recently isolated. On the other hand, if the strains are kept for different periods and then suspensions are prepared and examined all at one time against the necessary sera, it will be found that some of the strains do not agglutinate so well (if at all), as they did when first isolated. If, again, stock suspensions from the cocci are prepared immediately after isolation, it will be found that their agglutinability often increases as time goes on. Unfortunately, the extent to which both these changes may influence results is too irregular to be gauged. In these tests (Tables V and VI) the suspensions were generally prepared soon after isolation, and con-

TABLE V.

Cross-agglutination tests with 16 strains of *meningococcus* and nonordent sera prepared with seven cerebro-spinal strains and with two strains of cocci obtained from the naso-pharynx resembling the meningococcus in culture and morphology.

	M I	M IV	M VI	M XVI	M XIV	M XV	M XIII	NP 108	NP 235
	Agglutination at 1:100 Highest dilution with complete agglutination	Agglutination at 1:100 Highest dilution with complete agglutination	Agglutination at 1:100 Highest dilution with complete agglutination	Agglutination at 1:100 Highest dilution with complete agglutination	Agglutination at 1:100 Highest dilution with complete agglutination	Agglutination at 1:100 Highest dilution with complete agglutination	Agglutination at 1:100 Highest dilution with complete agglutination	Agglutination at 1:100 Highest dilution with complete agglutination	Agglutination at 1:100 Highest dilution with complete agglutination
M. i	c	c	c	±	±	±	±	c	c
M. ii	c	c	c	±	±	±	±	c	c
M. iii	c	c	c	±	±	±	±	c	c
M. iv	c	c	c	±	±	±	±	c	c
M. v	c	c	c	±	±	±	±	c	c
M. vi	c	c	c	±	±	±	±	c	c
M. vii	c	c	c	±	±	±	±	c	c
M. viii	c	c	c	±	±	±	±	c	c
M. ix	c	c	c	±	±	±	±	c	c
M. x	c	c	c	±	±	±	±	c	c
M. xi	c	c	c	±	±	±	±	c	c
M. xii	c	c	c	±	±	±	±	c	c
M. xiii	c	c	c	±	±	±	±	c	c
M. xiv	c	c	c	±	±	±	±	c	c
M. xv	c	c	c	±	±	±	±	c	c
M. xvi	±	c	c	±	±	±	±	c	±

c = agglutination complete.

± = agglutination well marked but incomplete.

±± = agglutination slight but definite.

sequently there was often considerable delay before agglutination tests with the different sera were carried out. These facts must be borne in mind in reading the results of the action of sera on naso-pharyngeal strains.

Table VI shows the action of the same set of sera on cocci isolated from the naso-pharynx. If this table be compared with the previous one giving the action of the same sera on meningococci of cerebro-spinal origin, it will be found that the results obtained with the naso-pharyngeal cocci can be roughly matched in a large proportion of instances with results obtained with the former. Very many match well with the results obtained with the group of meningococci represented by M I and M IV. There are a few, however, which give little or no agglutination with any of the sera tested.

In considering these results it must be remembered that agglutination with an anti-meningococcus serum does not necessarily imply close relationship with the meningococcus, since other organisms, *e.g.* the gonococcus, may agglutinate well with anti-meningococcus sera. On the other hand, a coccus cannot be definitely established to have no relationship with the meningococcus because it is not agglutinated by any of the sera of a particular series. Other sera might be found, if a sufficiently long search were made, which would agglutinate it well.

For purposes of comparison, in Table VII are given the results of the same set of sera on naso-pharyngeal cocci which could be distinguished by cultural or fermentation tests or both from the meningococcus. Many strains of the *catarrhalis* type are omitted owing to the impossibility of making a suspension suitable for the test.

Very few give any indications of agglutinability. No. 296 is an irregular organism which agglutinates with certain sera but also agglutinates completely with normal rabbit serum in a dilution of 1 : 50.

Absorption tests.

A positive absorption test with a given coccus is the best proof at present available that this coccus is identical with the meningococcus which produced the serum. The essential feature of the proof is the demonstration that, if a given dilution of an anti-meningococcus serum be brought in contact with a quantity of an unknown coccus which is just sufficient to remove the agglutinins that act on that coccus, it will also no longer agglutinate the homologous meningococcus. The test is carried out differently by different workers. I have followed as closely as possible the method proposed by Gordon and Murray (1915)¹.

¹ Identification of the Meningococcus, *Journ. Royal Army Med. Corps*, xxv, 411-423.

141	—	c	800	c	800	—	400	c	800	—	c	800	c	800	—	c	800
146	—	±	400	—	—	—	—	—	—	—	—	—	—	—	—	—	—
150	—	c	200	c	200	—	—	c	200	—	c	200	c	400	—	±	400
153	—	c	400	c	400	—	—	±	400	—	±	—	c	800	—	c	200
172	±	c	800	c	200	—	—	c	100	—	c	100	c	800	—	c	200
179	—	c	400	c	200	—	—	c	100	—	c	100	c	800	—	c	100
182	—	c	400	c	200	—	—	±	—	—	±	—	c	800	—	c	100
184	—	c	800	c	800	—	50	±	200	—	±	—	c	200	—	c	400
188	—	c	200	c	200	—	800	±	200	—	c	200	c	800	—	c	400
196	—	c	400	c	200	—	200	±	—	—	±	—	c	800	—	c	400
202	±	—	—	—	—	—	—	±	—	—	±	—	c	—	—	—	50
204	—	c	400	—	—	—	—	±	—	—	±	—	c	800	—	c	200
207	—	±	—	—	—	—	50	±	—	—	±	—	c	50	—	±	—
209	—	c	100	c	200	—	50	±	—	—	±	—	c	—	—	c	200
212	—	±	—	—	—	—	800	—	—	—	—	—	c	—	—	±	—
214	±	c	800	c	800	—	—	±	50	—	±	—	c	800	—	c	100
216	±	c	800	c	400	—	—	±	50	—	±	—	c	800	—	c	800
222	—	c	100	c	200	—	—	—	—	—	—	—	c	100	—	c	400
223	—	c	800	c	400	—	200	c	100	—	c	—	c	800	—	c	800
229	—	c	800	c	800	—	—	±	400	—	c	400	c	800	—	c	800
232	—	c	400	c	400	—	—	±	—	—	±	—	c	800	—	c	800
235	—	c	800	c	800	—	—	c	200	—	c	200	c	800	—	c	800
236	—	±	—	±	—	—	—	±	400	—	c	400	±	—	—	c	200
242	—	c	800	c	400	—	—	±	200	—	c	200	±	—	—	c	200
245	—	c	400	c	400	—	—	±	100	—	c	200	±	—	—	c	800
247	—	c	800	c	400	—	—	c	400	—	c	400	c	800	—	c	800
248	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	±	—
250	—	c	800	c	400	—	—	—	400	—	c	400	—	—	—	c	800
256	—	c	800	c	400	—	100	c	100	—	c	100	c	800	—	c	200
262	—	±	—	±	—	—	—	—	—	—	—	—	—	—	—	c	400
265	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	c	100
272	±	—	—	—	—	—	—	±	—	—	±	—	—	—	—	c	100

347	-	c	800	c	800	c	400	50	c	200	c	400	c	800	c	800	c	800
348	-	c	800	c	800	c	800	400	c	100	c	100	c	800	c	800	c	400
349	-	c	200	c	400	c	100	-	±	50	c	200	c	800	c	400	c	400
350	-	c	800	c	400	c	200	100	±	50	c	200	c	800	c	400	c	200
351	-	c	200	c	100	c	100	-	±	-	c	100	c	100	c	100	±	50
354	±	c	800	c	200	c	200	100	±	50	c	400	c	800	c	100	c	400
356	±	c	400	c	200	c	200	100	c	100	c	200	c	800	c	400	c	200
357	-	c	800	c	400	c	100	-	±	50	c	100	c	400	c	400	c	200
358	-	c	200	c	100	c	100	400	±	50	c	200	c	800	c	800	c	400
359	-	c	200	c	±	c	50	100	-	-	c	50	±	-	c	200	c	200
361	c	c	800	c	800	c	800	800	c	100	±	50	±	c	800	c	800	c
362	-	c	800	c	400	c	100	-	±	50	±	-	c	800	c	800	c	800
371	-	c	400	c	100	c	100	-	±	50	c	100	c	100	c	100	±	50
372	-	c	800	c	400	c	200	-	±	50	-	-	c	200	c	200	c	100
375	-	c	200	c	100	c	100	-	±	50	c	100	c	100	c	200	±	50
384	-	c	100	c	100	c	100	-	±	-	-	-	c	100	c	100	c	100
386	-	c	800	c	800	c	400	-	c	100	c	400	c	800	c	800	c	800
387	-	c	800	c	400	c	200	100	c	100	±	-	c	800	c	800	c	800
388	c	c	800	c	800	c	800	800	c	100	c	100	c	400	c	400	c	200
391	-	c	800	c	200	c	200	-	±	100	c	100	c	200	c	200	c	100
396	±	c	200	c	200	c	200	400	c	100	c	400	c	800	c	400	c	100
400	-	c	800	c	400	c	200	-	-	200	c	200	c	400	c	200	c	200

TABLE VII.
Agglutination reactions of strains which in sub-culture or in fermentation reactions were distinguishable from the meningococcus.

Strain	M I		M IV		M VI		M XVI		M XIV		M XV		M XIII		NP 108		NP 235		Differences from meningococcus sub-culture Colonies yellow in sub-culture Colonies yellow; fer- ments saccharose Colonies yellow; fer- ments saccharose Ferments saccharose Colonies yellow; fer- ments saccharose Colonies yellow; fer- ments saccharose Colonies yellow Colonies yellow Colonies yellow; fer- ments saccharose Ferments saccharose Unduly adherent to media Colonies yellow Ferments saccharose Colonies yellow Colonies yellow; ferments saccharose
	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination			
29	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow in sub-culture
60	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow in sub-culture
68	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow; fer- ments saccharose
90	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow; fer- ments saccharose
96	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Ferments saccharose
100	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow; fer- ments saccharose
39	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow; fer- ments saccharose
55	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow
64	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow
86	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow
237	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow; fer- ments saccharose
244	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Ferments saccharose
249	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Ferments saccharose
264	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Unduly adherent to media
276	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow
296	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Ferments saccharose
315	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	Colonies yellow

In this method the serum is diluted to 1 : 25 with saline, and an equal quantity of the standard suspension of cocci, as used in agglutination tests, is added: the mixture is then incubated for 24 hours at blood temperature. While this procedure was found to give satisfactory results with a few of my strains, I did not find that it worked well as a standard test, because in the case of many naso-pharyngeal strains the agglutinins acting on the test coccus were not removed to a sufficient extent, and this removal is an essential feature of the test. Gordon and Murray say: "should the result of an absorption test made in this way be at all doubtful, we then saturate the same serum over again and proceed as before. The first saturation sometimes clears off 'agglutinoids' very nicely from serum." It is evident that they have found the method with one "saturation" satisfactory only sometimes. If, now, as they state, a second "saturation" on the same serum is to be effected (presumably with the same standard suspension) it is difficult to see how they retain the same titration dilutions for the test, inasmuch as the serum must be diluted more than 1 : 100 when the final test is carried out.

To avoid this difficulty, and, while effectively removing the test coccus agglutinins, to employ as small an amount of the coccus in exhausting as possible, I adopted the plan of adding small quantities of a somewhat stronger suspension at definite intervals on three successive occasions, thus utilising the well-known fact that the same amount of suspension will remove agglutinins more effectively if it be added part at a time than if it be added all in one dose.

The details of the method were as follows: small centrifuge tubes were placed in series in a rack. Into each tube 2.5 c.c. of a 1 : 25 dilution of the serum used in the test was placed. Sufficient serum should be left over for use as a control in the unabsorbed state. A convenient number of tests to carry out at one experiment was found to be sixteen. For this number, 50 c.c. of a 1 : 25 dilution of the serum was made up: the 16 tubes required 40 c.c. and 10 c.c. were left over for testing in the unabsorbed state. The suspensions for saturating the serum were prepared in the usual way in the strength of 10 milligrams to the cubic centimetre, and were heated at 65° C. for an hour. Into the first test tube was then introduced 0.5 c.c. of the 10 mg. suspension of the organism homologous to the serum; into the next one or two was introduced 0.5 c.c. of similar emulsions of meningococci serologically similar to the homologous organism; into another tube was introduced 0.5 c.c. of an emulsion of an organism known to be inagglutinable with the serum; then into

the remainder of the tubes was introduced 0.5 c.c. of each of the strains of coccus to be tested. To the serum left over for controlling its effect in the unabsorbed state a similar proportion of phenol-saline was introduced, viz. one-fifth of its bulk. The tubes were then corked and placed in the incubator at 37° C. over night. The following morning the same process was repeated, and again in the evening; on the last occasion, when the final dose of emulsion was added, 1.0 c.c. of phenol-saline was also added to each tube, so that then the serum was fully diluted to 1 : 50. The serum for use in the unabsorbed state was also fully diluted in similar proportion. The titration was made on the following morning. Each of the tubes with suspension was centrifuged, and for each emulsion a rack was prepared containing ten agglutination tubes labelled *a* to *j* (see Table VIII). Into *a* was put 0.5 c.c. of the unabsorbed serum; into *b* was put 0.5 c.c. of the same serum diluted a half (now 1 : 100); into *c* and *d* were put the same amounts of 1 : 200 and 1 : 400 dilutions, respectively. Similar dilutions of the serum, after absorption by the coccus, were placed in tubes *e, f, g* and *h*. Into *i* and *j* were put the same dilutions as in *a* and *b*. The suspension used for the agglutination tests was prepared by diluting the stronger suspension used for absorbing, by adding four parts of phenol-saline to one of the strong suspensions, thus making a suspension of 2 mg. to the c.c. Into tubes *a, b, c, d, i*, and *j*, was put 0.5 c.c. of the coccus to be tested, and into tubes *e, f, g* and *h*, 0.5 c.c. of the suspension of the meningococcus homologous to the serum. The same process was carried out with the homologous meningococcus, the control cocci, and all the cocci under examination. The racks were then put in the incubator at 55° C. for 24 hours, and afterwards allowed to stand for 24 hours at room temperature before the results were read. In this method the serum is treated with 5 mg. of cocci in 3 c.c. of 1 : 30 dilution for about 15 hours, then with 5 mg. of cocci in 3.5 c.c. of 1 : 35 dilution for about nine hours, and finally with 5 mg. of cocci in 5 c.c. of 1 : 50 dilution for about 15 hours.

This procedure resulted in the satisfactory removal of the agglutinins acting on the test coccus in practically every instance. The amount of suspension used is very small, totalling 15 mg. of culture for 5 c.c. of a 1-50 dilution of serum or 3 mg. per c.c.

In Table VIII are given the results of absorbing M I serum with 30 naso-pharyngeal strains and five controls. These 30 strains all agglutinated with the serum when originally tested (Table I). Nineteen of them were obtained from the last 100 swabs (Nos. 301-400), which were taken from healthy workpeople at Cambridge. These are compared

TABLE VIII. Results obtained by absorbing an anti-meningococcal serum with 30 strains of cocci obtained from the naso-pharynx, compared with those obtained by absorbing the same serum with the homologous organism, other meningococci of a type similar to the homologous organism, and cocci of a different species.

		Serum M I												Result of Absorption
		Without absorption				After absorption								
		v. Test coccus				v. Coccus M I				v. Test coccus				
		A 1:100	B 1:200	C 1:400	D 1:800	E 1:100	F 1:200	G 1:400	H 1:800	I 1:100	J 1:200			
Homologous .	M I .	C	C	C	++	+	+	+	+	+	+	+	Complete	
Positive controls .	{ M IV .	C	C	C	+	+	+	+	+	+	+	+	"	
	{ M VI .	C	C	C	+	+	+	+	+	+	+	+	"	
Negative controls	{ Gonococcus	-	-	±	-	C	C	C	±	+	+	+	Negative	
	{ Flavus	-	-	-	-	-	-	-	-	-	-	-	"	
	{ NP 108	C	C	C	++	+	+	+	+	+	+	+	"	
	229 .	C	C	++	++	+	+	+	+	+	+	+	"	
	235 .	C	C	++	++	+	+	+	+	+	+	+	"	
	245 .	C	C	++	++	+	+	+	+	+	+	+	"	
	247 .	C	C	++	++	+	+	+	+	+	+	+	"	
	307 .	C	C	++	++	+	+	+	+	+	+	+	"	
	345 .	C	C	++	++	+	+	+	+	+	+	+	"	
	348 .	C	C	++	++	+	+	+	+	+	+	+	"	
	357 .	C	C	++	++	+	+	+	+	+	+	+	"	
	362 .	C	C	++	++	+	+	+	+	+	+	+	"	
	372 .	C	C	++	++	+	+	+	+	+	+	+	"	
	386 .	C	C	++	++	+	+	+	+	+	+	+	"	
	387 .	C	C	++	++	+	+	+	+	+	+	+	"	
	391 .	C	C	++	++	+	+	+	+	+	+	+	"	
	242 .	C	C	++	++	+	+	+	+	+	+	+	"	
	335 .	C	C	++	++	+	+	+	+	+	+	+	"	
	347 .	C	C	++	++	+	+	+	+	+	+	+	"	
	400 .	C	C	++	++	+	+	+	+	+	+	+	"	
	204 .	C	C	++	++	+	+	+	+	+	+	+	"	
	223 .	C	C	++	++	+	+	+	+	+	+	+	"	
	232 .	C	C	++	++	+	+	+	+	+	+	+	"	
	259 .	C	C	++	++	+	+	+	+	+	+	+	"	
	296 .	C	C	++	++	+	+	+	+	+	+	+	"	
	317 .	C	C	++	++	+	+	+	+	+	+	+	"	
	322 .	C	C	++	++	+	+	+	+	+	+	+	"	
	349 .	C	C	++	++	+	+	+	+	+	+	+	"	
	350 .	C	C	++	++	+	+	+	+	+	+	+	"	
	354 .	C	C	++	++	+	+	+	+	+	+	+	"	
	356 .	C	C	++	++	+	+	+	+	+	+	+	"	
	358 .	C	C	++	++	+	+	+	+	+	+	+	"	
Naso-pharyngeal strains													Complete or practically complete	
													Partial	
													Negative	

with ten strains from my Norwich cases (Nos. 201-300) and with one (No. 108) from the Bourn Tuberculosis Colony.

Special attention may be called to the strains from healthy work-people. It will be seen from the table that nine of them absorbed the agglutinin completely and four absorbed it partially. From this series of 100 swabs, therefore, 13 per cent. supplied a strain which answered every test for cerebro-spinal meningococci.

Very similar results were obtained by absorbing the serum M IV with the same series of cocci; these results are not shown.

Finally, the effect of saturating the two sera prepared from naso-pharyngeal strains was tested with three cerebro-spinal and several naso-pharyngeal strains. The results are shown in Tables IX and X, which may be considered together.

TABLE IX.

Results obtained by absorbing a monovalent serum prepared from a naso-pharyngeal strain with three strains of meningococci and fourteen strains of naso-pharyngeal cocci.

Strain	Serum NP 108									
	Without absorption				After absorption					
	v. Test coccus				v. NP 108 coccus				v. Test coccus	
	A 1:100	B 1:200	C 1:400	D 1:800	E 1:100	F 1:200	G 1:400	H 1:800	I 1:100	J 1:200
NP 108	c	c	c	±	±	±	-	-	±	±
M I	c	c	c	±	c	±	-	-	-	-
M IV	c	c	c	c	c	c	±	-	±	±
M VI	c	c	±	-	c	±	-	-	±	-
NP 235	c	c	c	±	c	±	-	-	±	-
307	c	c	±	-	c	c	±	-	±	±
322	c	c	±	±	±	±	-	-	±	±
335	c	c	±	-	c	c	±	-	±	-
345	c	c	c	±	c	c	-	-	c	±
347	c	c	c	c	c	c	±	-	c	±
348	c	c	±	±	c	c	±	-	±	±
350	c	±	±	-	c	c	c	-	-	-
357	c	c	c	c	c	c	±	-	±	-
362	c	c	c	±	c	c	±	-	±	±
372	c	c	c	±	c	c	±	-	c	±
386	c	c	c	±	c	c	-	-	±	±
387	c	c	c	±	c	c	-	-	c	±
391	c	c	c	-	c	±	-	-	-	-

TABLE X.

Results obtained by absorbing a monovalent serum prepared from a nasopharyngeal strain with three strains of meningococci, a strain of gonococcus and eleven naso-pharyngeal strains.

Serum NP 235											
Strain	Without absorption				After absorption						
	<i>v.</i> Test coccus				<i>v.</i> NP 235 coccus				<i>v.</i> Test coccus		
	A 1 : 100	B 1 : 200	C 1 : 400	D 1 : 800	E 1 : 100	F 1 : 200	G 1 : 400	H 1 : 800	I 1 : 100	J 1 : 200	
NP 235 .	c	c	c	±	±	—	—	—	±	—	
MI .	c	c	c	±	c	c	±	—	±	—	
M IV .	c	c	c	±	±	±	—	—	±	—	
M VI .	c	c	±	—	c	±	±	—	—	—	
Gonococcus	c	c	c	±	c	c	c	±	±	±	
NP 108 .	c	c	c	±	c	c	±	—	±	—	
307 .	c	c	c	±	c	c	±	—	±	±	
322 .	c	c	c	±	c	c	±	—	±	±	
345 .	c	c	c	±	c	c	±	—	±	±	
347 .	c	c	c	±	±	±	±	—	±	±	
348 .	c	c	±	±	c	c	±	—	—	—	
357 .	c	c	c	±	c	c	±	—	—	—	
362 .	c	c	c	±	c	c	±	—	—	—	
386 .	c	c	c	±	c	c	±	—	±	±	
387 .	c	c	c	±	c	c	±	—	±	—	
391 .	c	c	c	—	c	c	±	±	±	±	

With these sera it was found to be much more difficult to remove completely the agglutinin not only for the homologous strain but also for the test cocci, and it was found necessary to use for saturating 4.5 c.c. of the strong emulsion against 0.5 c.c. of the serum diluted 1 : 5; this is equivalent to 9 mg. of cocci per c.c. of a 1 : 50 dilution of serum. Even with this amount, as is shown in Table IX, the NP strains 345, 347, 372 and 387 left marked amounts of agglutinin for themselves as well as for the strain producing the agglutinin.

In such cases incomplete removal of the latter agglutinin may not indicate that the strain tested is specifically different from the agglutinin-producing strain, but it does indicate that a quantitative inferiority exists in the absorbing capacity of this tested strain as compared with others.

I have not met with the same phenomenon—difficulty in removing completely the agglutinin for the test coccus—among my sera prepared with strains of cerebro-spinal origin, but I am not prepared to erect this

into a general distinctive feature of sera produced with naso-pharyngeal cocci.

It will be seen, however, that the meningococci of pathogenic origin, M I, M IV, and M VI, removed a large portion of the agglutinin for the NP coccus used in producing the serum, as also did those NP cocci which, as shown in Table VIII, removed completely the agglutinin from M I serum.

It will be observed further that, though NP 108 and NP 235 are apparently identical with M I since they absorbed its agglutinin completely, yet they are not identical with each other since the agglutinins they produced varied in their combining capacity for different strains. For example, NP 322 absorbed from serum NP 108 almost all the agglutinin for NP 108 itself, whereas from serum NP 235 it absorbed relatively poorly. On the other hand NP 347 behaved in almost exactly the reverse manner, absorbing NP 235 agglutinin and leaving agglutinin in the case of NP 108. These two strains NP 322 and 347 differed from both NP 108 and NP 235 in failing to absorb the agglutinin for M I (vide again Table VIII).

Results such as these make it difficult to rely upon the absorption test for determining the specific identity of strains of unknown pathogenicity with known pathogenic strains. Positive absorption results may be regarded as unequivocal; but negative results, as in the case of NP 322 and 347, may, as just indicated, be quite compatible with relationship to a pathogenic strain and this relationship may be clearly demonstrable by the use of other sera.

SUMMARY.

1. In two areas in the Eastern Counties, Cambridge and Norwich, naso-pharyngeal swabs were taken from 400 individuals, who represented different conditions of health and social circumstances.

The investigation was made at Cambridge between the months of June and October, 1916, and at Norwich during August, 1916.

Owing to the low incidence of cerebro-spinal fever in the two towns during the year 1916 (see p. 249), the general population may be regarded as practically "non-contact" in respect of this disease.

2. As a result of the investigation, strains giving all the cultural and microscopical tests of the meningococcus were obtained as follows:

(a) From 200 swabs taken at Addenbrooke's Hospital during June and July, 1917, mostly from normal people, 30 strains.

(b) From 100 swabs taken at the Norfolk and Norwich Hospital during August, from individuals mostly in impaired health, 27 strains.

(c) From 100 swabs taken in Cambridge during October, from factory employees, who were all in good health and mostly in comfortable circumstances, 37 strains.

3. Such strains were found more often in males of every age group than in females, and in adults more often than in children.

In regard to the influence of health and surroundings, the results of the investigation grouped together show a larger proportion amongst town dwellers than amongst country people and amongst people with impaired health than amongst the healthy.

General conclusions cannot, however, be drawn from these figures because conditions were not always comparable. For instance, the majority of country people were examined in June and July, when positive findings were low.

In examining the employees at a factory, I obtained the strains in a higher proportion from the men working in those shops where air space was more restricted and ventilation less perfectly effected.

4. The 94 strains collected from 400 naso-pharyngeal swabs were tested as regards their agglutinability against certain monovalent sera. These sera were prepared from seven strains of meningococci of cerebro-spinal origin, and from two of the naso-pharyngeal cocci which had been isolated during the course of the investigation. The meningococcal strains used for preparing the sera included strains which had been found identical with others occurring with considerable frequency in cerebro-spinal fluid and capable of being grouped in two main groups; they also included strains which differed from these and were apparently rarer; but there is no reason to assume that every variety occurring in the specific disease was represented.

The results of the tests on the above 94 strains may be classified as follows:

(a) 22 gave no agglutination above 1 : 100 with any of the sera used.

(b) Two agglutinated well with certain sera, but also agglutinated with normal rabbit serum.

(c) 31 agglutinated up to 1-200 or 1-400 with the sera of a certain group (M I-M IV), and in some instances gave similar agglutination with other sera not belonging to this group.

(d) 39 agglutinated with the sera of the group M I-M IV to the full titre of the homologous strains, while with the sera belonging to other groups good agglutination was not often obtained.

This last group of 39 strains (41 per cent. of all tested) appears to be indistinguishable by simple agglutination tests from the M I-M IV group of cerebro-spinal meningococci. The previous group of 31 (33 per cent. of all tested) shows evidence of serological relationship with meningococci of cerebro-spinal origin, but it is not possible on their agglutination tests alone to give an opinion as to their identity with any meningococcal group.

Agglutination tests as a whole indicated that about 74 per cent. of strains collected on account of their resemblance to the meningococcus in culture, gave evidence of relationship to the meningococcus in virtue of their agglutination reactions with anti-meningococcal sera.

5. In order to determine if the absorption test would corroborate the relationship shown by agglutination, absorption tests were done against a serum prepared by inoculation of a cerebro-spinal strain of meningococcus, M I, with all the strains, culturally and by simple agglutination indistinguishable from pathogenic meningococci, which had been collected from the last 100 swabs (normal factory employees, Cambridge). Nine absorbed the agglutinin as well as the homologous coccus and four absorbed it partially.

Therefore, out of 100 normal people who had not been in contact with a case of cerebro-spinal fever, 9 per cent. were shown to be harbouring organisms in their naso-pharynx which were serologically identical with meningococci of pathological origin (cerebro-spinal fluid) and, in addition, 4 per cent., making 13 per cent. in all, were so closely allied as to be doubtfully distinguishable even by the test for absorption of agglutinin.

Similar results were obtained with strains obtained from Norwich.

The above results were confirmed by testing absorption by these cocci from another serum prepared with another spinal strain of meningococcus possessing properties almost identical with M I. I have not investigated absorption with sera prepared with strains which differ serologically from M I.

6. With regard to those naso-pharyngeal strains which were not identified with cerebro-spinal strains by serological tests (agglutination and absorption), I consider that in view of the great variation in the serological reactions shown by different strains of undoubted meningococci and even by different emulsions of the same strain, it is very difficult, if not impossible, to exclude any such microscopically and culturally typical organisms from the meningococcus group on the basis of serological tests.

AN ANOMALOUS MENINGOCOCCUS.

By R. G. CANTI, M.B. (CANTAB.).

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(With Plates II—IV.)

THE meningococcus which forms the subject of this paper has already been alluded to in this *Journal* (1917, vol. xvi. p. 249). The outstanding features then reported were:

(1) That the original cultures showed minute colonies, a very few of which subsequently grew to resemble typical meningococcus colonies in size and appearance.

(2) That subcultivations from the minute colonies, throughout repeated generations, behaved precisely as the original cultures.

(3) That subcultivations from the large colonies, throughout repeated generations, produced only large colonies.

It is proposed to set forth in further detail the observations which were made and to describe certain experiments which were undertaken with a view to determining the nature of this peculiarity.

LITERATURE.

There is little to be found in literature bearing upon this subject, although variations in the size of meningococcus colonies have frequently been observed. Elser and Huntoon (1909) noted that certain strains produced colonies as small as 1 and $1\frac{1}{2}$ millimetres in diameter throughout repeated subcultivations and maintained that this peculiarity was only partly due to the medium employed.

Kohlisch (1915) obtained from the cerebro-spinal fluid a culture which showed three kinds of colonies, differing from one another in their cultural appearance and made up partly of Gram-positive and partly of Gram-negative cocci. Subcultivations from one of these colonies yielded the same three kinds of colonies together with a fourth kind. From the knee joint he isolated an organism having certain of the same peculiarities.

One of the kinds of colonics isolated, both from the cerebro-spinal fluid and from the knee joint, was minute. Emulsions from each source were strongly agglutinated by antimeningococcal serum.

HISTORY OF THE CASE.

The organism at present under discussion was isolated from the meninges of K. aged 18 years, a railway porter who was admitted to St Bartholomew's Hospital on May 26th, 1916, under the care of Dr Horton-Smith Hartley, to whom I am indebted for permission to publish this note. Four days previously he had fainted but returned to work the following day. He was admitted, having had a fit, and when examined was in a dazed condition and complained of headache. There was no rash and no other signs or symptoms were present. Two days later he became delirious and the neck muscles were stiff. Lumbar puncture was performed, purulent fluid withdrawn and the current Lister Institute serum injected intrathecally. This was repeated the following day. Delirium in the meanwhile had ceased but returned after two more days, accompanied by violence. The patient then lapsed into coma and died, having been ill nine days in all.

A necropsy revealed a slight exudate over the base of the brain. There was no exudate over the vertex. The convolutions were slightly flattened, but there was no obvious dilatation of the ventricles. The cord showed a purulent exudate which was most marked on the posterior aspect in the thoracic region. This exudate was much thicker than that at the base of the brain. The heart showed a few haemorrhages beneath the visceral layer of the pericardium. The intestines showed much congestion of the mucous membrane especially in the upper part of the jejunum. Elsewhere no abnormality was found. A culture of the heart's blood was sterile.

BACTERIOLOGY.

Examination of films of the deposit of the cerebro-spinal fluid collected ante-mortem showed an abundance of polymorphonuclear cells and large numbers of Gram-negative diplococci which had the morphological appearance of meningococci.

Cultures were made on blood ascites legumin agar from each of the only two specimens of fluid withdrawn. After 24 hours' incubation at 37° C. the plates were covered with minute translucent colonies which appeared like those of the influenza bacillus. They did not exceed 0.2 mm. in diameter and were scarcely visible to the naked eye. A hand

lens showed them to be colourless and slightly raised with a sharply defined circular outline. Under the low power of the microscope fine granulations were present over the whole of each colony. Films stained by the Gram-fuchsin method showed them to be composed of Gram-negative diplococci having the usual appearances of meningococci. From 24 hours onwards for the next five or six days a marked alteration occurred to about 1 in 200 of the colonies. Each successive day on which the culture was examined it was found that here and there a colony had apparently taken on a new lease of life and had rapidly grown to a large size. Such a colony attained a diameter of about 2 mm. in the 24 hours after the commencement of this sudden increase, and 3 or 4 mm. in the next two or three days, by which time it had the appearance of a normal meningococcus colony having a faint yellow pigmentation in its centre.

In the meantime the bulk of the colonies, though growing slightly, remained minute, never attaining a diameter of more than 0.5 mm. Certain of these small colonies however, which happened to be near to the large colonies, themselves began to grow larger between 24 and 48 hours after the large colonies had become developed. These ancillary or secondary large colonies attained diameters up to 1.5 mm., and those which were nearest to the primary large colonies were larger than those more remote.

Subcultivations of the small colonies always behaved in the same manner as the original cultures throughout repeated generations for seven months, during which time the organism was carried through the single colony stage on more than 20 occasions. At the end of this time however the characteristics of the strain propagated became somewhat modified and the differentiation between the large and small colonies less conspicuous. The primary large colonies still appeared, but the secondary large colonies were rarely or poorly formed and the small colonies grew to a somewhat larger size and were less translucent.

Subcultivations from primary large colonies were always found to grow nothing but large colonies in 24 hours, and henceforward subsequent generations behaved like the typical meningococcus.

The same characteristics were found when small colonies were sown on to inspissated egg medium. After a week's incubation no more large colonies appeared but cultures were found to be alive after a month or more. By subcultivation from a mixture of small colonies on to legumin agar plates and then picking out a single small colony, the strain was kept going from time to time.

Ascites broth cultures of single small colonies 24 hours old, subcultivated on to legumin agar showed a pure growth of small colonies after 24 hours' incubation and subsequently behaved in the same way as cultures made direct from a single small colony.

The strain was submitted to Dr A. Eastwood who kindly made cultivations and found that the growth in Kutscher's agar had the same appearance as on legumin agar.

In the course of these investigations many batches of legumin agar were employed, and a large number of other meningococci cultivated on them showed, on all occasions, a vigorous and typical growth.

It might be argued that the nature of the large growing colonies was dependent upon the heterogeneous constitution of the medium, a large colony appearing at a spot where the medium possessed a quality particularly beneficial for its growth. But in view of the fact that the proportion of large to small colonies was the same on the various media employed, and on various batches of them, such a possibility can be excluded. Further this theory would not explain why the large colonies cropped up at more or less regular periods, for it would be expected that all the large colonies would appear at the same time.

ANALYTICAL OBSERVATIONS.

The table shows certain observations of the number of large colonies present on the plates after various periods of incubation. The first two columns show the figures obtained from the two original cultures of the cerebro-spinal fluid, and the remainder those obtained from subcultivations of the small colony line inoculated at various dates. The figures indicate the count of large colonies from day to day. Those plates on which the greatest number of large colonies appeared were observed to be the most heavily inoculated.

TABLE.

	May	May	May	June	July	August	August
24 hours' incubation	0	3	0	0	0	0	0
48 " "		36	19	9	4	17	9
72 " "		55	34	?	17		
96 " "			79	41	23		
120 " "			81		?		
144 " "					24		

A count was made on one occasion of both the large and small colonies and their relative numbers found to be 4700 and 24 respectively, that is approximately 200 to 1. During the months over which these

observations were carried on no noticeable alteration in proportion occurred.

It is seen that the large colonies increase in numbers more or less uniformly from day to day after the first 24 hours till about the fourth day after which time the appearance of a new large colony is rare.

Plates II—IV, Figs. 1—5, show successive photographs of a culture from a single small colony which had previously passed many times through the single colony stage. They were taken after 1, 2, 3, 4 and 6 days' incubation respectively, and are enlarged 1.75 diameters.

It is seen that in Fig. 1 the colonies are minute and that no large colonies are apparent¹.

In Fig. 2 primary large colonies are beginning to show themselves, and in Fig. 3 these have grown to a considerable size and more such have appeared. That these primary large colonies are due to the increase in size of small colonies and not to the appearance of new colonies is shown by selecting certain of the large colonies which happen to be in a thinly populated portion of the culture and tracing them back to earlier photographs where they are seen as small colonies identical with their fellows.

Fig. 4 shows the large colonies to have increased greatly in size and to exhibit a slight darkening in their centre. This is due to the faint yellow pigmentation to which reference has already been made. It is more apparent in the photographs than it was on the actual culture as the photographic plate employed was not "isochromatic." Around those primary large colonies which had existed as such for more than 24 hours, the groups of secondary large colonies are seen. Their gradual increase in size according to their situation is well demonstrated in those instances where they happen to lie in a straight line leading up to a primary large colony.

Fig. 5 shows the same changes in a more advanced stage. Incubation subsequent to this time showed no further increase in growth although the plates were sealed with paraffin wax to prevent evaporation.

Measurements have been made on the photographs with a view to ascertaining whether the centre of a newly formed large colony corresponds with the centre of the small colony from which it arose. It was found that whereas in some cases the two appear to be concentric yet

¹ These photographs are entirely untouched, and certain marks which might at first sight be mistaken for colonies are due to (1) flaws in the photograph which do not appear subsequently or (2) flaws in the agar medium which may be traced unaltered throughout the series.

in others the two centres undoubtedly do not coincide. This observation suggests that the sudden enlargement of the colony is not of a uniform nature, but it is due to a localized change commencing in some part of the colony, and possibly in one individual, a papilla being formed which rapidly overgrows the small colony and itself becomes paramount. If this is so the large colonies would appear to be analogous to the dulcitate fermenting papillae of *B. typhosus* and other similar papillae recently studied by Penfold (1911—1912—1913) with the difference that instead of many papillae appearing on one colony only one papilla was formed, and that on only a few colonies.

AGGLUTINATION.

Agglutination tests were kindly carried out by Professor Andrewes on two emulsions each prepared from cultures of single small colonies and on two emulsions each prepared from single large colonies. All four emulsions were agglutinated to approximately the same titre by Gordon Type II serum (Gliddon) and not with Type I or Type III sera.

A rabbit serum was prepared from an emulsion of small colonies and was found to agglutinate the homologous coccus and other cocci of Type II to a high titre.

CARBOHYDRATE REACTIONS.

Glucose ascites litmus broth tubes sown with organisms from each kind of colony were fermented in three days. Similar cultivations in saccharose were not fermented in one week.

ADJUVANT BROTH EXPERIMENTS.

The observation that those small colonies in close proximity to the large colonies themselves increased in size suggested the probability that the medium surrounding the primary large colonies became modified by them in a manner beneficial to the small colonies. Accordingly the following experiment was carried out.

A primary large colony was selected from a young culture of a line of small colonies which had passed through the single colony stage three times since isolation. It was inoculated into an Erlenmeyer flask containing about 200 c.c. of broth mixed with fresh sterile ascites fluid. A similar flask of uninoculated ascites broth was also prepared as a control. Both flasks were incubated for 23 days at 37° C. The inoculated broth (which on cultivation gave a pure growth of meningococci) was

then filtered through a Berkefeld filter under aseptic conditions and the filtrate incubated for a few days to prove its sterility. Two "stab" tubes each containing 16 c.c. of legumin agar were then melted and cooled to 45° C. To one of them was added 4 c.c. of the filtrate and to the other 4 c.c. of the control broth. Plates were then poured from these mixtures and each was inoculated with a loopful of a 24 hour ascites broth culture from a single small colony of a line which had passed through the single colony stage on twelve previous occasions. The plate containing the filtrate showed after 24 hours' incubation a copious growth of normal sized colonies, among which there were many small colonies in the more crowded areas. After 48 hours' incubation there was a general increase in the size of all the colonies although a fair number of rather small colonies still remained. On the control plate the culture showed the usual characteristics of the strain. There was a profuse growth of minute colonies at the end of 24 hours and after a further 24 hours nine colonies had attained the size of a normal 24 hour old meningococcus colony.

The experiment was repeated and a similar and even more striking difference was observed between the filtrate culture and the control.

Plate IV, Figs. 6 and 7, shows photographs, enlarged to 1.75 diameters, of the two cultures of the first experiment taken after 48 hours' incubation.

It is thus seen that legumin agar containing 20 % of the filtrate of a broth culture prepared from a large colony markedly increases the growth of colonies of the small growing line.

DISCUSSION.

The large colonies which are formed appear to be identical with typical meningococcus colonies. They have been observed to spring from an atypical strain in which the colonies formed were minute. In this respect the strain is a rarity.

According to De Vries (1906) evolution takes place by three kinds of steps, progressive, retrogressive, and degressive. The first of these steps is achieved by the acquirement of a new character, the second by the loss or latency of a character, and the third by the reappearance of such a latent character. Now if the forward or positive step produces a variety which is identical with a variety commonly found and further produces it from an uncommon variety, it seems permissible to assume that the newly formed variety arose by the reappearance of a character latent in its ancestors and is therefore degressive. In this case the coccus

producing the small colonies would be of a retrograde or negative variety having arisen by the latency or loss of a character which reappears in certain of its progeny by a degressive step, the common variety being again evolved.

With regard to the nature of the latent character which reappears, the adjuvant broth experiments show that the large colonies produce a substance which when mixed with the nutrient medium will cause a culture of the small colony strain to develop much larger colonies than on the nutrient medium alone. It would therefore appear that the character latent in the cocci which produces small colonies is the capacity to produce a substance beneficial for their growth. The existence of such a substance has been suggested in connection with bacterial "lag" as the possible reason why maximal growth does not occur in broth cultures of *Bacillus coli* till some time after incubation has been commenced: the idea, however, is not consistent with all observations recorded. (Penfold, 1914.)

The ancillary relation of one kind of organism to another where the former grows with greater vigour in the neighbourhood of the latter is a well recognised fact, and in nature generally it is frequently observed that two individuals of different kinds may live in proximity for their mutual benefit. Further it is generally supposed that individuals produce substances which are always useless and frequently inimical to their own growth and that of their kind. The above experiments, however, seem to indicate that in at least some cases a living individual produces a substance which is necessary for its well being, an observation which is totally contrary to general experience. What the nature of this substance may be is a matter of conjecture. Consideration must be given to the possibility of the formation of acid by the large colonies till a hydrogen ion concentration is attained which is optimum for growth. That the substance in question belongs to the "vitamine" class seems improbable since the original cultures were made in a medium containing fresh blood in which presumably the necessary "vitamines" were present in sufficient quantity. It however seems admissible to assume that the organisms in the large colonies may be capable of producing a ferment or of otherwise breaking down the too highly complex nutritive material in the medium, and may thus prepare for themselves a substance which they are capable of assimilating. In this case the adjuvant broth would contain this substance which has been formed during its incubation in excess of the requirements of its population, and would then be able to supply nutritive material in the required form to those cocci which



Fig. 1.



Fig. 2.



Fig. 5.



Fig. 6.



Fig. 7.

lacked the capacity of converting the more highly complex substance to their use.

SUMMARY.

(1) An unusual strain of meningococcus has been isolated which produces minute "dew drop" colonies. This character has persisted throughout a large number of successive generations. Every culture, however, has shown lateral off-shoots consisting of colonies which have the size and appearance of those of typical meningococci. In subsequent subcultivations from these colonies the newly acquired size and appearance remain constant.

(2) It is suggested that the strain isolated is a retrograde variety and that the off-shoots are produced by degressive evolution.

(3) It is also suggested that the character which reappears in the degressive step is that of producing a substance beneficial for growth.

It is impossible, however, to offer any satisfactory explanation of the phenomena observed without a more extended series of experiments. This was impracticable at the time owing to pressure of routine work and further observations on the same organism have now become impossible owing to the fact that the small growing strain has died out. Nevertheless the findings seem worth recording in their present state in the hope that they may be of use to others who have met or may meet with similar peculiarities in the course of their work.

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IDENTIFICATION OF THE MENINGOCOCCUS.

By M. H. GORDON, C.M.G., M.D.,

Temp. Hon. Lieut.-Colonel R.A.M.C.

(With 1 Text-figure.)

I. PRELIMINARY RESEARCHES.

During the years 1903 and 1904 while engaged upon an investigation of the micro-organisms present in saliva, I had frequent occasion to make a quantitative bacteriological examination of this material. The object then in view was to define the bacteria that are most numerous in the mouth, so as to see if they could be applied to detect particulate pollution of air by material derived from the upper respiratory passages in a way similar to that in which *B. coli* is used to detect and measure excremental pollution of water. While certain streptococci were found to provide the index in question, particularly *S. salivarius* (which is constantly present to the extent of 10 to 100 millions per cubic centimetre of normal saliva) it was observed that among other bacteria present in the cultures were certain gram-negative cocci which frequently exceeded 100,000 per c.c. of the saliva of normal individuals.

In 1905 in view of the recent manifestation of cerebro-spinal fever abroad in epidemic form and the discovery that this disease is spread by persons who carry the meningococcus in their nasopharynx, it appeared desirable to define the characters of these gram-negative cocci of normal saliva more closely, so that should occasion arise hereafter the information might be available for identifying carriers of the meningococcus.

Accordingly, an investigation of these gram-negative cocci was undertaken, and it was soon obvious that they were of several different kinds. As my object was to sort them, and previous experience with streptococci had shown that this was most likely to be achieved by a study of their fermentative characters, representative specimens of the gram-negative cocci were isolated and cultivated in a slightly

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alkaline medium containing one or other of some sixty carbohydrates, polyatomic alcohols, or glucosides. As a result it was found that glucose, maltose, saccharose and galactose were of value for differentiating these salivary gram-negative cocci from one another, and by means of their ability to break up one or other of these carbohydrates 127 gram-negative cocci derived from human saliva were resolved into five separate groups. To make the investigation complete, the meningococcus and gonococcus were also examined with regard to their behaviour in these tests, and the points in which they differ from each other and from the gram-negative cocci of normal saliva defined.

In addition to the difference in respect of fermentative characters, it was found that the question of growth or no growth on agar or nasgar at 23° C. was a further point of practical value for the purpose of differentiating these gram-negative cocci from the meningococcus which, as Albrecht and Ghon first showed in 1901, does not grow on ordinary media below a temperature of 25° C.

While this investigation was at an early stage, I was invited by Dr R. A. Dunn, M.O.H. of East Herts, to co-operate with him in an investigation of a mysterious illness of an infectious nature which had appeared in the district for the health of which he was responsible. This illness was at one time suspected of being cerebro-spinal fever, and in course of the bacteriological investigation of material from the nasal passages of persons affected by it, my collection of gram-negative cocci became considerably augmented. The chief micro-organism found in the nasal secretion of the Herts case was *M. catarrhalis*, but among other gram-negative cocci encountered were some that resembled the meningococcus more closely than the majority of those which I had previously come across in cultures from normal saliva. On proceeding to compare certain of these cocci with the meningococcus it was discovered that even when the sugars previously mentioned failed to differentiate them, the majority were clearly distinguishable from the meningococcus by their ability to ferment mannose; moreover all of them were distinguished from it by the fact that they grew readily upon nasgar at 23° C.

Up to the time of this investigation, attempts had been made with variable success by several observers, to apply the agglutination test for the purpose of differentiating the meningococcus. My own efforts in this direction were not of an encouraging nature, and therefore I was unable to recommend the test at this stage of the research. Looking back, it is easy to see now that two of the chief reasons of this lack of

success were, first that I employed the microscopic method only, and secondly that the most satisfactory way to prepare agglutinin for the meningococcus had not then been determined.

The conclusion reached, therefore, was that the gram-negative cocci of normal saliva were of several different kinds, and the characters of most value for differentiating them from the meningococcus were the appearance of their growth on agar or nasgar at 37° C., their ability to grow on nasgar at 23° C. to 25° C., and their action upon glucose, maltose, galactose, and saccharose respectively and, in certain cases, mannose. The value of these fermentation tests for the purpose in view was subsequently confirmed by others, notably by von Lingelsheim whose results appeared in the year following that of the publication of the paper by Dunn and myself.

Shortly after this investigation had been completed, I was fortunate enough to secure the co-operation of Dr T. J. Horder in an experimental investigation of the protective value of various samples of antimeningococcus serum. It is unnecessary to describe this investigation further here than to remark that we found that whereas the total growth of as much as six young slope cultures on nasgar of the particular meningococcus with which we were working could be injected intravenously in a single dose into a rabbit without fatal effect, nevertheless a total of but four to six of such cultures was invariably fatal to the animal if given *seriatim*, and an hour's interval was allowed to elapse between the injection of each individual culture. The experience then gained with this method of saturation proved of very great value some eight years later.

II. MEASURES ADOPTED FOR IDENTIFICATION OF THE MENINGOCOCCUS ON THE MANIFESTATION OF CEREBRO-SPINAL FEVER AMONG TROOPS IN TRAINING DURING 1915.

In the early months of 1915 cerebro-spinal fever broke out among recruits in training, a large epidemic was threatened, and special measures became imperative for limiting the spread of this disease. The sanitary measures adopted for this purpose were directed by Colonel W. H. Horrocks, C.B., K.H.S., who was assisted by Surgeon-Colonel R. J. Reece, C.B., and I was invited by the Medical Research Committee to advise with regard to bacteriological matters, and also to carry out research. The following procedure was adopted. On occurrence of a case of cerebro-spinal fever, the patient was removed to an isolation hospital for treatment, and the contacts were segregated and swabbed.

Each contact whose nasopharynx yielded no meningococcus-like organisms was returned to duty with the smallest possible delay, while those whose nasopharynx was found to contain an organism resembling the meningococcus were kept in isolation until the characters of the suspect coccus had been further determined. Thus it was insured that contacts whose nasopharynx yielded an organism indistinguishable from the meningococcus were kept in isolation until two successive nasopharyngeal swabs taken at an interval of several days proved negative.

A Central Laboratory was set up at the R.A.M. College and District Laboratories were started or co-opted for the purpose of dealing with this disease in military districts throughout the country. A special department in the Central Laboratory was instituted for the manufacture and supply of media to the laboratories and placed under the charge of Major T. G. M. Hine. For the purpose of obtaining material from the nasopharynx for bacteriological examination, the covered swab introduced by Mr C. E. West, F.R.C.S. was adopted.

From the bacteriological point of view, the immediate need was for a practical and rapid method of identifying the meningococcus in the nasopharyngeal secretion, while it was essential that the procedure adopted should be of such a nature that the bacteriologists who were detailed or co-opted for this work—most of whom had little or no previous experience of identifying the meningococcus in nasopharyngeal secretion—could readily carry it out with the appliances of an ordinary bacteriological laboratory.

In the memorandum which was issued in February 1915 giving instructions as to the measures to be taken on the occurrence of a case of cerebro-spinal fever, and the mode of passing the nasopharyngeal swab, the following procedure was prescribed for identification of the meningococcus.

“Secretion from the nasopharynx. The stages of the investigation are as follows.

“(1) Examination of separate colonies on the cultures. Colonies of the meningococcus appear at 37° C. in 24—48 hours. They are larger than colonies of the accompanying pneumococci and streptococci, they are clear, smooth and transparent, have a firm outline, and are very characteristic. A portion of one of these colonies taken up on a platinum needle is found to emulsify readily in a drop of water on a glass slide.

“(2) Gram's Stain. A film made from one of these colonies shows gram-negative diplococci.

• “Subcultures are made and placed at 37° C. and at 23° C. respectively.

The meningococcus does not grow at 23° C., whereas the vast majority of the gram-negative cocci of normal saliva grow readily at this temperature.

"In view of the sharp distinction which this test provides, it will be sufficient for practical purposes to regard the suspicious cocci that have passed it as meningococci.

"Confirmatory tests should be employed for greater accuracy as follows:

"*Fermentation Tests.* The meningococcus ferments glucose with the production of an acid reaction, but fails to change saccharose. These tests are applied by making subcultures at 37° C. in media tinted with litmus and containing the above sugars respectively.

"*Agglutination.* The meningococcus shows positive agglutination when brought in contact with anti-meningococcus serum."

This scheme of identifying the meningococcus in nasopharyngeal secretion had the advantage of simplicity and speed; moreover it was found to work quite smoothly in practice. The majority of the contacts were found to harbour no meningococcus-like organisms, and were returned to duty within 48 hours.

It was plain, however, at the outset that the bacteriological procedure was of a provisional nature only, and that while it made practical application of the knowledge then available for the exclusion of the commoner gram-negative cocci with which confusion was likely to arise, there was reason to suspect the possible existence of a further group of these organisms indistinguishable from the meningococcus in the particular characters submitted to scrutiny, but without the same significance in regard to epidemic cerebro-spinal fever.

The history of bacteriology in its application to medicine shows repeated instances of the confusion of specific pathogenic bacteria with others closely resembling them in morphological, cultural, and sometimes even in fermentative characters also, but entirely devoid of the same pathogenic significance. The earliest instance in which this similarity led to error appears to have been brought about by the resemblance between the anthrax bacillus and *B. subtilis*. The similarity between the diphtheria bacillus and certain diphtheroid bacilli is another example that has certainly led to mistakes; and the resemblance in morphological and cultural characters between the cholera vibrio and some other vibrios found in nature has undoubtedly caused difficulty in the past. The history of the attempts to identify the typhoid bacillus in

water is a further and striking illustration of the need of caution before resting satisfied that a given micro-organism is identical with one of specific pathogenic importance solely because of a close resemblance in morphological, cultural, and fermentative characters.

Accordingly, it was realised at the outset that the procedure adopted for recognising the meningococcus in nasopharyngeal secretion merely represented the best that could be done in the circumstances, and that the only way to define and remedy its possible defects was by intensive research. This research was rendered still more urgent by the military necessity of holding up no man unless he carried a meningococcus of known epidemic significance.

III. DEFINITION OF THE MENINGOCOCCUS OF THE OUTBREAK BY MEANS OF THE AGGLUTINATION TEST.

In view of the work that had been done by Jochmann, Von Lingelsheim, Lieberknecht, Kutscher, Dopfer, Netter and Debré, Elser and Huntoon, Raymond Koch and others, and the improvements in knowledge and technique that had resulted from their labours, it was anticipated that the agglutination test would prove of immediate use for the purpose of identifying the meningococcus. On trial, however, it was found that specimens of the anti-meningococcus serum then available failed to agglutinate meningococci isolated from the cerebro-spinal fluid of our cases.

ANALYSIS OF MENINGOCOCCI OCCURRING IN THE CEREBO-SPINAL FLUID OF THE CASES.

Before an agglutinating serum could be obtained, therefore, that would serve for the purpose of identifying the micro-organism of the outbreak, it was necessary to start *ab initio* and first of all to collect meningococci from the cerebro-spinal fluid of the cases and to define them by the agglutination test. It was clear also from the work of Lieberknecht, Dopfer, and Elser and Huntoon that the absorption test should be applied as well in order to check the results of simple agglutination.

The steps of this investigation have been described in detail in the *Journal* of the R.A.M.C., and also in a report to the Medical Research Committee. The first requirement was to obtain agglutinating serum of good quality in as short a time as possible. By means of the saturation method to which reference has been made, it was found that excellent agglutinating serum could be prepared from young rabbits within ten

days. Meningococci were collected from the cerebro-spinal fluid of thirty-two cases of the disease and systematically investigated by Captain E. G. Murray and myself in respect of their agglutination reactions and the results checked by the absorption test. In view of the work of Kutscher and Lieberknecht, the macroscopic method was used and the tubes examined after 24 hours at 55° C. From the first the method was standardised. Raymond Koch's discovery that a suspension of meningococci would keep for several months in saline and serve quite well for agglutination tests provided it is heated in the first place for half-an-hour to 65° C. and 0.5 % of phenol then added as a preservative, was made use of; and all suspensions standardised to contain the same number of meningococci by a turbidity test that was worked out for this purpose. In order that our results might be checked by others, full particulars have been given of the *procédure* employed.

As the outcome of this investigation the thirty-two meningococci were found to be resolved into four different groups as follows:

Type	1	2	3	4
Specimens	19	8	4	1

As a rule the results of simple agglutination were confirmed by those given by the absorption test. There were in some cases though not in all close affinities between members of Types 1 and 3, and 2 and 4; but absorption tests proved that these affinities were due to minor or co-agglutinins, and that the major agglutinin of each of the four types was univalent and specific. Complete cross tests and controls were carried out with all thirty-two cocci against each of the four univalent sera.

IV APPLICATION OF THIS INFORMATION FOR THE PURPOSE OF IDENTIFYING THE MENINGOCOCCUS IN NASOPHARYNGEAL SECRETION.

Gram-negative cocci from the nasopharynx of nine contacts and one doubtful case of cerebro-spinal fever were now submitted to investigation with the four univalent agglutinating sera that had been proved to include all of the serological types of meningococcus occurring in the cerebro-spinal fluid of the thirty-two cases. Each of these ten nasopharyngeal cocci was indistinguishable from the meningococcus in morphological, cultural, and fermentative characters. As a result of the test, six of these nasopharyngeal cocci were found to be serologically identical with the meningococcus—five being specimens of Type 2 and

one of Type 1. The remaining four nasopharyngeal cocci could not be identified serologically with any of the four types of meningococcus. In order to test the matter further, two specimens of these four pharyngococci were injected into rabbits and an agglutinating serum prepared against each of them. The specific agglutinin of each of the pharyngococci while readily removed by the homologous coccus, was quite unaffected by any of the four types of meningococcus obtained from the cerebro-spinal fluid of the cases. The two pharyngococci in question also appeared to be serologically distinct from one another. Thus the suspicion mentioned previously was confirmed and the existence established of a group of pharyngococci indistinguishable from the meningococcus in the morphological, cultural and fermentative characters submitted to examination, but nevertheless distinct serologically from any of the types of meningococcus present in the cerebro-spinal fluid of the thirty-two cases of cerebro-spinal fever.

V. ROUTINE ADOPTION OF THE AGGLUTINATION TEST FOR THE PURPOSE OF IDENTIFYING THE MENINGOCOCCUS IN CASES OR IN CONTACTS.

During the autumn of 1915 further specimens of meningococcus from the cerebro-spinal fluid of cases were submitted to scrutiny with the four univalent sera. For this purpose Major Arkwright generously supplied me with cultures from the collection which he had made during preceding stages of the outbreak, and Dr O'Brien did the same. As a result, by the end of 1915 over sixty specimens of meningococcus had been examined with all four agglutinating sera, and found to be identical with one or other of the four serological types.

As soon as the types of meningococcus present in the outbreak had been defined, cultures of them were forwarded to those who prepare anti-meningococcus serum with a note as to their frequency. In order also to make the identification of the meningococcus more accurate it was decided to provide all District Laboratories with the necessary materials for determining the type present either in the cases or in contacts. Major Hine carried out a research in which he defined the relative value of various modes of dosage for the purpose of obtaining agglutinating serum for the meningococcus in the shortest possible time, and by this means elaborated an intensive method which has now been in continual use at the Central Laboratory for over two years with uniform success. The manufacture and supply of univalent agglutinating sera and emulsions was taken over by him and these were

supplied to District Laboratories in outfits, the expense of which was defrayed by the Medical Research Committee. With each outfit the following directions were issued:

CEREBRO-SPINAL FEVER.

AGGLUTINATION TESTS.

Directions for applying the agglutination test to meningococci and meningococcus-like organisms with the outfit supplied for this purpose from the Central Cerebro-Spinal Fever Laboratory, R.A.M. College, S.W.

20th December, 1915.

Investigation of meningococci occurring in the recent outbreak of cerebro-spinal fever among the troops in this country has shown that the majority of specimens of this micro-organism isolated from the cerebro-spinal fluid of the cases, although alike in cultural and fermentative characters, are differentiated by the agglutination test into one or other of three main types.

The relative abundance of each of these types up to the present stage of investigation of the recent outbreak is seen from the following figures. The number of specimens of meningococci from cerebro-spinal fluid examined was sixty-one:

Type	Specimens	Percentage
1	31	50
2	20	32
3	10	16

In addition to these three predominant types, several other types of meningococcus have been differentiated by the same means, but the latter, owing to their comparative rarity so far, appear to be relatively unimportant from the point of view of controlling the epidemic among troops.

The above facts have a practical bearing both on treatment, and also on identification of the meningococcus in the nasopharynx of carriers.

SERUM TREATMENT OF THE PATIENT.

In order to ensure that the correct specific serum is given, it is desirable to prepare a suspension of the particular meningococcus occurring in the cerebro-spinal fluid of the case, and to determine its type by the agglutination test.

IDENTIFICATION OF THE MENINGOCOCCUS IN THE NASOPHARYNX OF CARRIERS.

According to present evidence, cerebro-spinal fever is chiefly spread by carriers. In order to check the spread of this disease, therefore, it is desirable to detect and isolate any persons carrying in their nasopharynx known epidemic strains. Recognition of such strains of this micro-organism is now possible by means of the agglutination test.

METHOD RECOMMENDED FOR APPLYING THE TEST.

The following articles are required:

1. Specific agglutinating sera univalent for each of the three chief types of meningococcus occurring in the cerebro-spinal fluid of cases during the present outbreak, and a sample of normal serum for use as control.
2. Four sterile test-tubes for making dilutions of these sera.
3. A fine calibre pipette holding 0.5 c.c. and graduated to 0.1 c.c.
4. A 5 c.c. pipette graduated in 1 c.c. and 0.1 c.c. divisions up to the point.
5. Small test tubes 3" by $\frac{1}{2}$ ", sterilized and plugged with sterile wool.
6. A stand to hold these tubes. (See below.)
7. Fresh sterile saline (0.85 per cent.).
8. Standardized and phenolated suspensions of each of the meningococci homologous to the three specific sera, and similar suspensions of the several cocci to be tested.

When identifying the meningococcus in cultures from the nasopharynx, the first stage is the selection of meningococcus-like colonies in the plates after twenty-four hours growth at 37° C. A colony sufficiently typical for further test having been carefully selected, the steps for carrying out the agglutination test are as follows:

PREPARATION OF STANDARD SUSPENSION OF SELECTED COCCUS.

As the method recommended is the macroscopic one, it is necessary in the first place to obtain enough growth of the coccus to make a fair quantity of the suspension. The coccus, therefore, should be spread over the surface of two or more legumin agar plates or plates of other suitable medium. After twenty-four hours incubation at 37° C., the growth on each plate is washed off in 5 c.c. of saline with the aid of a sterile wire or a glass rod bent to an angle, then poured into a sterile test tube and well shaken. Film preparations are next made of these suspensions, stained with Gram, and examined for purity. Meantime the tubes have been placed in a water bath at 65° C., at which temperature they are allowed to remain for thirty minutes in order to kill the coccus and to destroy its autolysin.

STANDARDIZATION OF THE SUSPENSION.

This is effected in the following way. With a pipette delivering 0.1 c.c., this amount of the suspension is transferred to an ordinary clean $\frac{3}{4}$ -in. test tube specially kept for this purpose. Clear tap water is then run in from the 5 c.c. graduated pipette until the contents of the tube are only just—but still definitely—turbid by daylight when compared with the control tube of tap water. This end-point is taken to represent a content of 100 millions of the coccus per cubic centimetre. A simple calculation then gives the number of cocci contained by the suspension. For example if 0.1 c.c. of the suspension requires to be diluted to 8 c.c. with tap water to reach the end point, 0.1 c.c. of the suspension, therefore, contains 800 million cocci; and 1 c.c. of it 8,000 million. Supposing there are 5 c.c. of the suspension; this contains, therefore, 40,000 million cocci altogether. Now it has been found by experiment that a suspension of meningococcus containing 2,000 million per c.c. gives excellent results as regards macroscopic agglutination. In the present example, then, there are sufficient

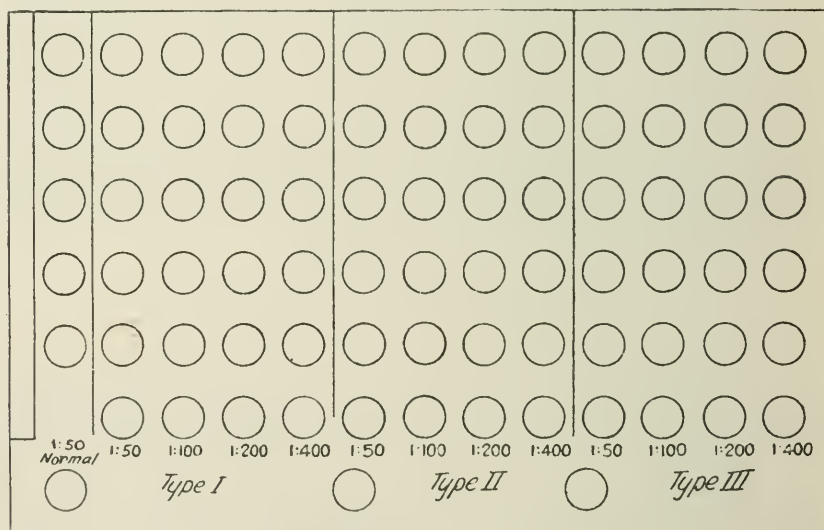
cocci to make 20 c.c. of such a standard suspension. Accordingly the 5 c.c. of suspension is poured into a sterile measuring glass, diluted with saline up to 18 c.c., and then 2 c.c. of a 5 per cent. solution of phenol in distilled water is added to it as a preservative. The suspension, heated, standardized, and phenolated in this way is poured into a bottle, labelled and dated. It has been found that such suspensions of meningococci keep for several months.

The standardized suspensions having been prepared in this way, and the other articles being at hand, procedure is as follows:—Let us imagine, for example, that five gram-negative cocci are to be tested with the agglutinating sera prepared against the three chief types of meningococcus that have been obtained from the cerebro-spinal fluid of epidemic cases.

ARRANGEMENT OF TUBES IN THE STAND.

This stand is designed to hold enough tubes to test five different cocci at the same time against the three specific sera with the necessary controls.

In order to determine the degree of agglutination, the coccus is put up against each of these three sera in four dilutions. To prove that the three agglutinating sera are active, a control is also put up of each of them against its homologous coccus in the same dilutions. Thus eight cocci in all are tested, three known and five unknown. As an additional control, each coccus is also put up against a sample of normal serum.



Plan of Agglutination Board.

$\frac{3}{8}$ Scale.

The stand consists of a wooden block with six rows of thirteen cylindrical holes to contain the agglutination tubes. On the left is a xylonite strip for labelling each row. The holes are marked out in three blocks, stained amber, white, and blue respectively; each one of these coloured blocks contains twenty-four holes, and is

assigned to a separate specific serum. In addition there is a vertical row of five holes labelled "Normal," and there are also three single holes at the bottom for controls of normal serum against the three "standard" cocci.

The dilutions of the several sera are first of all filled into the tubes in vertical rows; then the suspensions of the different cocci are added in turn to the horizontal rows.

Thus each of the three sera has a block of four vertical rows, and a single row is reserved for the normal serum on the left. Each coccus of test has one horizontal row which runs through all three blocks of specific sera starting with the row devoted to normal serum.

The standard suspensions and anti-sera issued are put up in amber, white and blue bottles and capsules for Types I, II and III respectively, corresponding to the three blocks of holes in the board. There is also a green capsule containing the normal serum.

EXAMPLE OF TEST.

The following example will illustrate the procedure employed in further detail. The titre of all the three specific sera used is supposed to be 1 : 400. It is proposed to test the cocci against them in a dilution of 1 : 50, 1 : 100, 1 : 200, and 1 : 400 respectively.

SERUM DILUTION.

(a) Normal serum (control).

0.2 c.c. of normal serum is placed in a test tube by means of the small 0.5 c.c. pipette and 4.8 c.c. of saline added. From the 5 c.c. of 1 : 25 dilution of normal serum thus obtained, half cubic centimetre amounts are distributed in the first left-hand vertical row of five tubes, and the three single tubes below them.

(b) Agglutinating serum for "Type I" meningococcus.

A 1 : 25 dilution is made of this serum in the same way as in the case of the normal serum, but as more of it will be required in order to furnish the larger number of dilutions necessary, 0.3 c.c. of this serum is taken in the first place and diluted with 7.2 c.c. of saline. Half cubic centimetre amounts of this 1 : 25 dilution are then run into the six tubes of the first left-hand vertical row of Type I block (amber). In order to convert the remainder of this dilution in the test tube into a 1 : 50 dilution, 4.5 c.c. of saline are added to it, and half cubic centimetre amounts of this are then distributed in the second vertical row of six tubes. The procedure is repeated in the same way for the next two rows, but as the volume of the dilution that is left in the tube steadily increases it is advisable to reject some of it after row two, so that the quantity can be easily contained in a test tube of ordinary size, when diluted with an equal volume of saline. As the result of this series of dilutions, the four vertical rows of block I contain half cubic centimetre amounts of Type I serum in dilutions of 1 : 25, 50, 100 and 200 respectively.

The procedure is then repeated with the agglutinating sera for meningococcus Types II and III, and successive dilutions of these distributed as before in half cubic centimetre amounts in the tubes of blocks 2 (white) and 3 (blue). All the tubes have now a dilution of the required serum in half the final dilution decided upon.

The next step is to fill in from the standardized suspension bottles half cubic centimetre amounts of each of these respectively into each tube in the horizontal

rows with the 5 c.c. pipette including the control tubes in the left-hand row containing normal serum. Finally four half cubic centimetre amounts of each of the control cocci homologous to the specific sera are filled into groups of four tubes in the lowest horizontal row of the stand. All the tubes are then plugged and incubated for twenty-four hours at 37° C., or 55° C.

RESULTS.

In reading off the results it is advisable to always examine the tubes in the same order. Thus the controls of each coccus with normal serum in the left-hand vertical row and three bottom single tubes are examined first, and then the controls of homologous cocci in the lowest horizontal row. The former being negative and the latter positive; the degree of agglutination of each coccus to the three specific sera respectively is next noted. It is convenient to have one's own signs for indicating the degree of agglutination. Thus “+” signifies absolutely clear fluid and the cocci clumped in flocculi at the bottom of the tube; “++” indicates obvious flocculi of fair size floating in the fluid, but the latter still somewhat turbid; “(+)” indicates turbid fluid with small flocculi only.

If the controls are not satisfactory no inference can be drawn. Thus unless agglutination of the control type coccus takes place with its corresponding anti-serum, there is no guarantee that the specific agglutinin is active. Similarly, unless the controls with normal serum are negative, the results with specific sera are of no account. It should be remembered that certain examples of *M. flavus* agglutinate with normal serum.

The titre of 1:400 is chosen for this experiment because it is a good average one. Some sera go higher, in which case the primary dilutions would, of course, be correspondingly increased.

GROUP AGGLUTININS.

It has been found that certain gram-negative cocci of the nasopharynx other than meningococci may agglutinate with these anti-meningococcus sera in their low dilutions. Such of these cocci, however, as have been met with do not agglutinate beyond the first, or the two lowest, dilutions; they have been found by absorption tests not to absorb the specific agglutinin from these univalent sera.

In order to avoid confusion from the action of group agglutinins, this differentiation of meningococci occurring in the cerebro-spinal fluid of cases in the present outbreak into three main types has been effected by controlling the result of the agglutination test by an after-test to determine whether the specific agglutinin has been absorbed or not. Strictly speaking, the agglutination test furnishes presumptive evidence only: complete identification is not established until it has been proved that the specific agglutinin of the type meningococcus has been absorbed by the coccus of test. For practical purposes, however, the agglutination test appears to be sufficient; provided that it is conducted quantitatively, and the titre of the coccus of test compares with that of a control of the homologous coccus with the same serum at the same time. It should be mentioned that Types I and III appear to be closely related, meningococci of Type I frequently, but not always, showing some agglutination with Type III anti-serum.

GENERAL REMARKS.

It will be clear from the above that when cerebro-spinal fever breaks out in a district, it is most important to test the meningococcus occurring in the cerebro-spinal fluid of the case or cases in order to determine its type. The information obtained in this way is a guide both to the correct specific serum for therapeutic use, and also to the type of meningococcus to be specially looked for in the nasopharynx of contacts. It would seem desirable to keep a watch in the same way on meningococci occurring in the cerebro-spinal fluid of any further cases in order to see if the predominant type should alter; in which case a corresponding modification would be necessary in the defensive measures. Again, it will be of great interest to know whether more than one type of meningococcus is to be found at the same time in the cerebro-spinal fluid of a case of cerebro-spinal fever; and also whether the same type occurs in the nasopharynx as is present in the cerebro-spinal fluid of the patient.

The Medical Research Committee have arranged to supply, free of cost to medical officers specially appointed for the study and treatment of military cases of cerebro-spinal fever, sets of the necessary outfit, with standard agglutinating serum and standardized homologous agglutinable suspensions of each of the three types respectively, as part of the assistance they have given to the War Office in the scientific study of measures for the treatment and control of the disease.

All applications for the outfit, with standard sera and suspensions, should be addressed to the

CENTRAL C.S.F. LABORATORY,
ROYAL ARMY MEDICAL COLLEGE,
GROSVENOR ROAD, LONDON, S.W.

It will be observed that owing to its rarity up to that time, Type 4 was at first not included in the outfit. A few cultures of meningococcus were received about this period from sporadic cases of meningitis among children and could not then be identified. In view of later experience, and especially of Captain Tulloch's research, it is highly probable that at any rate some of these were really specimens of Type 2. To provide for the possible appearance of new types, or of types not previously identified, officers in charge of District Laboratories were asked to forward to the Central Laboratory any meningococci from cerebro-spinal fluid that refused to agglutinate with the sera sent out.

During the following months the disease recrudesced and the agglutination test was found to furnish much valuable information. The cocci received as not agglutinating with the sera were comparatively few, and the majority of them proved to be specimens of Type 4. It also was found that in the case of Type 2 some sera were better than others owing to the tendency of certain specimens of this type to become sub-typical in culture. In order to exclude error from this cause freshly isolated specimens of Type 2 were used to make the antiserum of this

type. In all cases only a single meningococcus has been used at a time, and throughout only meningococci actually isolated from the cerebro-spinal fluid of cases have been employed in the preparation of agglutinating serum.

Owing to the appearance of Type 4 in a proportion of the cases during an outbreak in a large garrison in the early part of 1916, this coccus and its antiserum were added to the outfit from that date. With one exception, however, no further outbreaks have come to notice in which this particular type of meningococcus has been at all frequent.

Now that an accurate method of identifying the meningococcus in the nasopharynx was available, the following memorandum was sent round to District Laboratories with the object of obtaining further information concerning the general distribution among the military population of meningococci of known epidemiological significance.

“MEMORANDUM.

(1) MODE OF SPREAD OF CEREBRO-SPINAL FEVER. EXAMINATION OF CONTROLS.

As part of the investigation of factors governing the spread of cerebro-spinal fever, it is very desirable that, as far as possible, opportunity should be taken of making control observations with a view to ascertaining what proportion of non-contacts harbour the meningococcus in their nasopharynx.

It is suggested that, other things being equal, it would be more useful to examine for this purpose small groups of men from a large number of units, rather than a large number of men from the same unit.

Before such investigations are undertaken, a particularly careful enquiry should be made to ascertain whether or no there has been any possibility of recent contact with a case of cerebro-spinal fever, or with a carrier from such a case.

No gram-negative coccus should be accepted as the meningococcus for the present purpose unless it fails to agglutinate with normal serum, and at the same time agglutinates to approximately the same titre as the homologous meningococcus with one or other of the anti-meningococcus sera supplied.

In positive cases it will also be valuable to know (1) the relative abundance of the meningococcus in the nasopharyngeal mucus of the person carrying it, and (2) the duration of such carrying.

(2) DEFINITION OF THE RELATION BETWEEN OUTBREAKS OF
INFLUENZAL CATARRH AND CEREBRO-SPINAL FEVER.

In some camps severe outbreaks of coughs and colds have occurred. It is desirable to make observations on the bacteriology of these cases both from the point of view of the presence or absence of the meningococcus, and also for the purpose of determining what are the prevalent bacteria in these cases of catarrh."

CENTRAL C.S.F. LABORATORY,
ROYAL ARMY MEDICAL COLLEGE,
GROSVENOR ROAD, LONDON, S.W.
February 16th, 1916.

VI. RESULT OF PRACTICAL APPLICATION OF THE AGGLUTINATION
TEST FOR IDENTIFICATION OF THE MENINGOCOCCUS.

The report of Captain Martin Flack on cerebro-spinal fever in the London District during 1916 demonstrates in a convincing manner the value of the agglutination test in actual practice, both for detecting cases of the disease, and also for identifying carriers. With regard to cases he found (1) that not more than a single type of meningococcus could be obtained from the cerebro-spinal fluid of a patient, (2) on examination of the nasopharynx of the patient he confirmed the later observation of von Lingelsheim as to the constant presence of the meningococcus there at the onset of the disease, and he showed further that this meningococcus in the nasopharynx was always of the same type as that present in the cerebro-spinal fluid of the patient when this was positive. A similar observation as to the identity of type between the meningococcus in the nasopharynx and cerebro-spinal fluid of the same case has also been reported by Major F. W. Andrewes. This constant presence of the meningococcus was found by Captain Flack to have a direct clinical application in facilitating a correct diagnosis of cerebro-spinal fever in the very cases where help of this kind was most needed, namely in cases where the symptoms of the patient were atypical, or where the cerebro-spinal fluid failed to yield a growth of the meningococcus. The importance of early diagnosis cannot be over-emphasised from the point of view of successful serum treatment. Captain Flack also found that the same type of meningococcus was most prevalent both in cases and in carriers during the period that he was in charge of the London District C.S.F. Laboratory.

Another observation made by him was that, like the cases, chronic carriers are remarkably monotypical in the sense that the great majority of them carry one and the same type of meningococcus throughout the whole period of their carrying. This was confirmed by Captain Tulloch.

A good opportunity for comparing the types of meningococcus prevalent in cases and in carriers respectively occurred during an outbreak of cerebro-spinal fever in a large garrison during 1916.

Meningococci from the cerebro-spinal fluid of thirteen cases of the disease were examined at the Central Laboratory with the following results:

Type	1	2	4
Specimens	3	5	5

A very large number of men in this garrison had been swabbed by Captain R. R. Armstrong and amongst them he found a proportion who harboured suspicious gram-negative cocci in their nasopharynx. From 193 of these men Captain Tulloch obtained cultures of gram-negative cocci that agglutinated with one or other of the univalent agglutinating sera. The distribution of types among these men was as follows:

Type	1	2	4
Specimens	30	72	71

The correspondence of types in cases and carriers is remarkable. It may be added that the identification of the types in the cases and carriers was made independently, and that it was not until Captain Tulloch came to write up his report that this closeness of the grouping in the two series was noticed.

For the last two years monotypical agglutinating sera have been in routine use at the Central Laboratory and in District Laboratories for detecting the meningococcus both in cases and in carriers with satisfactory results, and it is hoped that some of the reports of District Laboratories bearing upon this matter will be published. Meningococci isolated from troops coming from England, Scotland, Wales, Ireland, France, Gallipoli, Australia, New Zealand, Canada, and South Africa have been submitted to examination and in the vast majority of instances relegated with ease to one or other of the four types. When preparing a new circular for issue with the agglutinating outfit during August of the present year it was found on inspecting the records that during the past two years meningococci from the cerebro-spinal fluid of over 300 cases of the disease had been tested in the Central Laboratory alone, with the result that approximately 98 per cent. of them had been

identified by the agglutination test with one or other of the four types. The relative abundance of individual meningococci has varied from time to time, but on the whole their relative frequency may be put roughly as follows:

Type	1	2	3	4
Frequency	40 %	45 %	10 %	5 %

Thus while 85 % of the cases have been due either to Type 1 or to Type 2, the experience of 1915 has been confirmed throughout each of the two years that have elapsed since the types of meningococcus at work in the present outbreak were defined.

VIII. THE RESULT OF INVESTIGATION OF FURTHER SPECIMENS OF THE MENINGOCOCCUS BY THE ABSORPTION TEST.

With a view to checking previous results, and also to obtaining further information concerning the types of meningococcus occurring in cases of cerebro-spinal fever, Captain W. J. Tulloch continued the investigation of these organisms by means of the absorption test. In this way he examined with scrupulous care 100 more specimens of meningococci from the cerebro-spinal fluid of cases. Captain Tulloch's paper on this subject has been published in the R.A.M.C. *Journal* for July 1917. His investigation not only confirmed previous results, but also brought out a new and very important point, namely that in the case of meningococci belonging to Type 2—previously the most difficult of all to classify by the absorption test—no less than three distinct sub-groups are distinguishable. This sub-grouping in the case of Type 2, however, does not interfere with the practical utility of the agglutination test when identifying Type 2, for Captain Tulloch has shown that by preparing a rabbit with a sufficiently typical meningococcus of this type an agglutinating serum can be prepared that includes cocci of all its three sub-groups by the agglutination test. Captain Tulloch's research has thus been of the greatest value not only in consolidating information upon this most important matter, but also in improving the accuracy of the method in its practical application.

INTER-RELATION OF THE TYPES.

Are the four serological types of the meningococcus merely temporary variants of one and the same micro-organism, or are they pathogenically distinct members of the same group, somewhat after the manner of *B. typhosus* and *B. paratyphosus a* and *b*?

The answer to this question given by the absorption test is undoubtedly to the effect that the types are not temporary variants, but are distinct and stable entities. In order to throw further light upon the matter the following experiments have been carried out.

SUPERIMPOSITION TESTS.

On considering this problem of the relationship of the types of meningococcus to one another it appeared that in addition to the information afforded by the absorption test, further information might be gained by comparing the effect of injecting the meningococcus of another type into a rabbit already elaborating agglutinin in response to previous stimulation with a given type of meningococcus. If the second type of meningococcus were only a temporary variant of one and the same micro-organism as the first, then the effect of superimposing it in this way should be merely to stimulate the production of the agglutinin which the animal was already in course of elaborating. If, on the other hand, the type secondarily injected were a specifically different micro-organism then the first agglutinin so far from being increased, would exhibit its normal decline; and a new agglutinin specific for the new antigen would make its appearance in the rabbit's blood.

These experiments have been described elsewhere. In the first place five young rabbits were all injected with Type 1 and when on the sixth day their blood showed a titre of from 1:300—1:600 for this coccus four of the rabbits received a second injection. Thus rabbit A was not interfered with: rabbit B received a second dose of Type 1, rabbit C received a dose of Type 2, rabbit D of Type 3 and rabbit E of Type 4. The result was that in the case of the rabbit that received the second dose of Type 1, the original agglutinin was increased, whereas in the case of each of the other three rabbits a new agglutinin specific for the new type, and previously absent from the rabbit's blood made its appearance. Further experiments in which Type 2 was first injected and Type 3 and 4 superimposed showed that in such cases also while the agglutinin for the primary coccus declined, new agglutinins appeared in the rabbits' blood specific for the cocci of Type 3 and 4 respectively in a precisely similar fashion as had been observed in the first experiment. Finally the superimposition of Type 4 in a rabbit already elaborating agglutinin to Type 3 resulted in the same way in the birth of a new agglutinin specific for Type 4 in the rabbit's blood.

These superimposition experiments therefore confirm the result of the absorption tests in a very definite manner, and afford striking evidence of the serological specificity of the different types.

IMMUNITY TESTS.

In order to define the protective values of prophylactic inoculation of individual types of meningococcus, a group of rabbits was prepared against Type 1, another against Type 2 and other groups against Types 3 and 4 respectively. After each animal had received a number of doses at weekly intervals and showed a good yield of homologous agglutinin in its blood, one rabbit of each group and a control normal rabbit were saturated with Type 1, another set with Type 2, and the same repeated with Types 3 and 4. As a result it was found that in the case of Types 2, 3, and 4, the homologous rabbit survived, while the other rabbits succumbed. The protection afforded by prophylactic inoculation, therefore, with these types was univalent. In the case of Type 1, on the other hand, the homologous rabbit in spite of its previous treatment was no more protected against Type 1 coccus than a control normal rabbit. The whole experiment was repeated with new specimens of the type cocci and a similar result obtained.

So far as they go, therefore, these immunity tests indicate that in the case of Types 2, 3 and 4, the protection is mainly univalent. In the case of Type 1, it is clearly far more difficult to protect a rabbit than in the case of the others, a difficulty probably due in great part to the particularly potent endotoxin of this type.

It may be here mentioned that Kennedy and Worster Drought have drawn attention to a point observed by them with regard to the relative intensity of the illness in cases of cerebro-spinal fever, according as they are infected by one or another type of meningococcus. In their experience cases due to Type 1 are far more severe and fatal than most of the cases due to Type 2. This clinical experience appears to be in accord with what has been observed elsewhere, and is on a par with the result of the protective tests described above. Some severe outbreaks due to Type 2, however, have occurred. Fulminating cases may be produced by any of the types.

It may be of interest to mention here that a particular therapeutic serum of which much use was made during the early stages of the outbreak in 1915, with disappointing results, was tested at the time against meningococci from the cerebro-spinal fluid of the cases and found not

to agglutinate them. The coccus then most prevalent was Type 1. A sample of this serum was recently submitted for examination and tested against the four types with the result that while agglutinins for 1 and 2 were practically nil, agglutinins for 3 and 4 were found to be abundant. The inference that the horse furnishing the serum had been prepared against the wrong types is difficult to avoid. The interest of this observation, however, is not limited to the negative virtues of the serum. The only medical officer who has obtained good results with this serum (and whose request for more led to my examining the sample) is one in whose district Type 4 cases have certainly been identified, and also some cases in which the infecting coccus could not be identified with Type 1 by absorption tests and almost certainly was an example of Type 3.

The following instance of the value of monotypical serum is only a single case, but it is worthy of attention. A young woman of good physique was seized with cerebro-spinal fever, and as the serum which she received did her no good, the civilian Medical Officer in charge sent an urgent request for another brand. Having some Rockefeller serum kindly sent to me by Dr Flexner for trial, I forwarded some with the request that a sample of the patient's cerebro-spinal fluid should be submitted so that the type of meningococcus present could be determined. This material shortly arrived and the coccus was found to be a specimen of Type 3. The sample of Rockefeller serum, though strong in agglutinin for Types 1 and 2, showed very little for 3 and 4. I was not surprised therefore to hear that the Rockefeller serum had been no more successful than the other. Now we had a few bottles of Type 3 serum made by Dr Stanley Griffith for us a year previously when we were trying to get agglutinating serum from horses for identification of types of meningococcus. The horse serum proved useless for the purpose then in view, although it had a high titre, for Type 3 co-agglutinins were numerous in the lower dilutions. This serum was the patient's only hope, and it was therefore sent for trial. The effect, it is pleasing to report, was extremely satisfactory; the patient making a rapid recovery forthwith. This monotypical serum was also used on some other Type 3 cases about the same time with satisfactory results, although the routine serum had failed to benefit them.

Measures are being taken at the present time to prepare monotypical sera on a larger scale for more extensive trial in the treatment of cases of cerebro-spinal fever.

CONCLUSION.

The agglutination test controlled by the absorption test, therefore, has proved practically of the greatest possible use in dealing with the outbreak of cerebro-spinal fever among the military forces. By its means a serious error has been eliminated when identifying carriers of the meningococcus; diagnosis has been facilitated—particularly in those cases where it was most needed; and the meningococci occurring in the cerebro-spinal fluid of cases have been differentiated into four separate types which breed true and while closely similar in characters of minor importance, are clearly distinguished from each other by the reaction to them of the tissues of the living animal.

The full results of this differentiation have still to be reaped, but a severe practical trial of the agglutination test during the past two years on a scale that is without precedent in this country, has demonstrated beyond all reasonable doubt that this method constitutes at the present time by far the most valuable of all known methods of identifying the meningococcus.

APPENDIX.

NOTE ON SOME RECENT OBSERVATIONS BY OTHER WORKERS
WITH REGARD TO THE CLASSIFICATION OF MENINGOCOCCI.

During the early stages of the present outbreak, Arkwright and Ellis observed independently that meningococci occurring in the cases, fell into two distinct groups. Both observers relied upon simple agglutination.

Prior to the present outbreak, the work of Arkwright in this country and of Dopter in France had paved the way for the present differentiation. Arkwright made a collection of meningococci isolated from cases and tested their agglutinative characters. By this means he obtained evidence of the presence of several different kinds of them, and he noticed that diversity was more marked in case of meningococci from sporadic than in those from epidemic cases. The *Comptes Rendus* from 1909—1914 contain a set of papers by Dopter that are of special interest, because they show the stages by which, from an entirely independent standpoint, this distinguished Medical Officer of the French Army arrived at a conclusion very similar to that reached by us as the result of a systematic serological analysis by the agglutination + absorption test of meningococci from military cases during the present outbreak. In the first place his researches on the gram-negative cocci from the nasopharynx of soldiers led to the identification of a group of cocci

indistinguishable from the meningococcus in morphological, cultural, and fermentative respects, but distinct in agglutinative characters and in the absorption test. To this group he gave the name parameningococcus. On proceeding to examine meningococci from the cerebro-spinal fluid of cases of cerebro-spinal fever in the same way, he found that serologically two different kinds could be distinguished. The first he regarded as the meningococcus, the other he called parameningococcus. The investigation of meningococci from the cerebro-spinal fluid of further cases led to the definition in the same way of still further parameningococci until by May, 1914, he had distinguished no less than three parameningococci all serologically distinct from the meningococcus and from one another.

M. Nicholle of the Department of Serotherapy of the Pasteur Institute has received specimens of our four types of meningococcus, and regarded Types 1 and 3 as belonging to the meningococcus group, and 2 and 4 to the group of parameningococcus. I understand that he has not applied the absorption test: without this of course the action of co-agglutinins cannot be excluded.

In a volume recently issued by the Medical Department of the Local Government Board, certain criticisms are made of the procedure with reference to identification of meningococcus summarised in the present paper, and an attempt is made to throw doubt upon the value of the absorption test. The work thus criticised, however, is its own witness; and the demonstrated success of the quadrivalent agglutination test in practice during the last two years for identifying the meningococcus both in cases and in carriers among the forces is sufficient to dispel these doubts based to a large extent upon theoretical considerations, which, as Captain Tulloch shows, are extremely insecure from the scientific point of view.

Definition of the relationship between pharyngococci and meningococci is a matter of research rather than of philosophy: research moreover of a distinctly arduous character. The final criterion so far has been the capacity of a given coccus to combine with a specific agglutinin *in vitro*. Now in its practical application to gram-negative cocci the absorption test upon which the final decision rests is work demanding a very high degree of dexterity that can only be acquired by continuous and persevering effort. The test is a process of balancing, dependent upon a series of very accurate measurements and quantitative adjustments; and even minute errors may mar or upset the result. In our experience at the Central Laboratory it requires at least

six weeks hard work before even a trained bacteriologist with considerable serological experience can sufficiently master the technique to obtain consistently satisfactory results in absorption tests of these delicate micro organisms. Similarly when he goes for a holiday—even for a week—it requires at least another week's work before the necessary unconscious manipulative dexterity returns. After that degree of skill is reached, irregular results are far less frequent than before. Granted that this factor of technique is equal in both sets of workers, the following sources of fallacy merit attention.

(1) When we were working out the meningococci of the present outbreak by the absorption test, the urgency of reaching a decision in the shortest possible time saved us from a pitfall into which the Local Government Board workers may have fallen. In addition to the quality of the antigen, a factor of first-rate importance in the absorption of agglutinin test is the quality of the agglutinin used. Now it was observed at an early stage of the investigation that our sharpest cut results with the absorption test were obtained with the "First-Born" agglutinin: that is to say the agglutinin that appears first in the rabbit's blood in response to injection. While strictly specific, this agglutinin is in our experience more ready to unite with the antigen employed than older agglutinin. The agglutinating serum used by us throughout came from young rabbits (1000 grams) which had all received intensive treatment. Most of them had not been under preparation for more than ten days before their blood was collected. The serum from rabbits immunised over several months is not in our experience the best for absorption tests.

A concrete instance may be given to illustrate the importance of the quality of the agglutinin in this test. During 1915 a univalent agglutinating serum prepared against Type 1—which as a rule is the easiest to work with—had been employed on a number of occasions for absorption tests, and was promptly returned to the cold store when not in use. After several months, however, the agglutinin in this serum—while still agglutinating its type cocci practically as well as before—was found to have lost much of its power of combining with the specific antigen, and the serum had to be replaced by another one freshly prepared. This gave the same sharp-cut results that we had been accustomed to.

In this relation it may be mentioned that in our experience horses' serum is not suitable for absorption tests with gram-negative cocci. For some reason, the agglutinin in the serum of horses appears to be

less ready to combine with these organisms *in vitro* than the agglutinin made by the young rabbit. It should be added, however, that our experience in this matter has been limited to specimens of antimeningococcus serum supplied for therapeutic use, and that these specimens of horse serum were of some standing.

(2) Another conceivable reason why the results of these workers are not in harmony with our own is the possibility that the difficulties of Type 2 (perhaps the most widely diffused type of all) which Captain Tulloch succeeded in overcoming by an arduous research did not yield to their attack.

(3) A third possible reason—and, in view of Arkwright's observations on the matter, this is the most probable explanation of all, is that serological diversity amongst meningococci from cases in children of the civil population—from whom much of their case-material appears to have been derived—is greater than in that of meningococci obtained from soldiers during an epidemic. My present experience of them, though small, has certainly led me to suspect that meningococci from sporadic cases in children are serologically less uniform, and therefore more difficult to define, than those obtained from military cases during an outbreak of cerebro-spinal fever.

That the difficulties, however, of a complete classification of meningococci are by no means insuperable, is indicated by the following facts.

(1) In a letter received from him in July last Colonel C. J. Martin wrote, "I think I told you that when I was at home last winter we tested out upwards of 100 strains which had been kept going at the Lister, and found that all but one would fall comfortably into one or other of your four groups."

(2) Captain Pullon, Government Bacteriologist at Capetown, investigated a series of meningococci obtained from cases of cerebro-spinal fever in South Africa, and having differentiated them with agglutinating sera prepared by himself, forwarded suspensions of these cocci to the Central Laboratory, for examination. The suspensions arrived a few weeks ago and were tested against the four univalent sera in the routine manner with the result that of sixteen meningococci from cerebro-spinal fluid, nine were specimens of Type 1, five were specimens of Type 2, and two were specimens of Type 3. No example of Type 4 was found. On reference to the enclosure in Captain Pullon's letter giving his own results, it was seen that the classification of these cerebro-spinal fluid strains effected by our sera was identical with that made by him; the only difference being that his group A was our Type 3,

his Group B, Type 2, and his Group C, Type 1. There is good reason to believe, therefore, that a limit obtains with regard to the diversity of meningococci, and that at any rate the most important pathogenic members of the group have now been defined.

(3) Since the above was written, Staff-Surgeon P. Fildes and Surgeon S. L. Baker, R.N., have published in *The Lancet* of January 19th, 1918, a paper on "The Grouping of Meningococci into Types." Full particulars are given of the reactions shown by meningococci isolated from 46 cases of cerebro-spinal fever at Haslar to the agglutination and absorption test with four univalent sera prepared independently by themselves. The conclusion reached is as follows. "Gordon's statement that practically all meningococci capable of producing cerebro-spinal fever are found to belong to one of his four groups is confirmed by our results. It therefore follows that an unknown coccus from the throat or elsewhere which does not belong to one of these four groups is not likely to be a pathogenic meningococcus. This rule may be taken to be invariable for practical purposes."

A STUDY OF THE MECHANISM OF THE AGGLUTINATION AND ABSORPTION OF AGGLUTININ REACTION, TOGETHER WITH AN EXAMINATION OF THE EFFICACY OF THESE TESTS FOR IDENTIFYING SPECIMENS OF THE MENINGOCOCCUS ISOLATED FROM 354 CASES OF CEREBRO-SPINAL FEVER.

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At the present time owing to the difficulties arising from war conditions, and the stress of work due to the special circumstances in which the study of bacteriology is being prosecuted, we have had in some cases to modify established methods of bacteriological procedure and introduce new technique.

The difficulty of obtaining an adequate supply of pure carbohydrates for performance of sugar tests and the even greater difficulty of obtaining men trained in laboratory work in bacteriology has hastened this change in general procedure to a very considerable extent. It is important that the nature—so far as this is susceptible of examination in the present state of our knowledge—and limitation of procedures which have in the past been commonly made use of, or have recently been introduced, be carefully scrutinised.

Such scrutiny would serve the useful purpose of preventing undue enthusiasm for, and its converse, unmerited condemnation of, methods which when correctly appreciated and properly carried out afford valuable information.

In the following paper the first three sections are devoted to a consideration of the mechanism and limitations of the agglutination phenomenon, and of the absorption test. The last section (4), is occupied with an analytical survey of the actual result of application of the agglutination test—checked in a large proportion of the cases by the absorption test—to over 300 specimens of meningococcus isolated from the C.S. fluid of cases and examined at the Central Laboratory, up to the present time.

In order fully to appreciate the potential errors of the agglutination phenomenon, and its amplification, the absorption of agglutinin test, in their application to the identification of micro-organisms and the establishment of the relationship which these bear to disease processes, it is essential that the factors concerned in the mechanism of these reactions should be borne in mind.

Our knowledge of these reactions has progressed considerably of recent years, and I think that we may fairly state now that the views of Ehrlich in respect of the phenomenon under consideration are no longer tenable. The same may be said of the theories advanced by Gruber and Durham, Nicolle, Paltauf and Dineur, to explain the clumping of micro-organismal suspensions when exposed to the action of corresponding anti-sera.

The view advanced by Bordet that the process of agglutination occurs in two distinct phases, viz. (1) the union of antigen with antibody, (2) the flocculation of the united antibody antigen "couple" by the electrolytes of the fluid in which the reaction is carried out, has never been controverted, but, on the contrary, has been corroborated by all the experimental work carried out since Bordet's original discovery.

I shall, therefore, discuss the problem of agglutination from the standpoint of Bordet.

In the process of agglutination three separate systems react with one another, the antigen, the antibody and menstruum in which these are suspended; and the reaction is divisible into two phases of (1) union of antigen with antibody, (2) flocculation of the antibody-antigen complex.

I. POINTS TO BE NOTED CONCERNING THE UNION OF ANTIGEN WITH ANTIBODY.

(a) *Influence of the reaction of the menstruum in which the reagents are suspended.*

Two of the interacting substances—the antigen and the antibody—are, so far as is known, of the nature of complex colloids. The complexity of these colloids is further enhanced by the fact that the active bodies are probably only constituents of still more complex systems. The conditions, therefore, which determine the union, or interaction, of antibody with antigen are not so far susceptible of complete investigation. Nevertheless, there is evidence that this union occurs only under certain definable conditions. Thus in the case of organisms of the

colon-typhoid group at least it will not take place if the fluid be too acid or too alkaline, i.e. the interaction of the two "active" colloids is determined to some extent by the reaction of the menstruum and it appears to take place most readily when the suspending fluid is isoelectric with the mixture of colloids.

The following simple experiments illustrate this point:

To investigate the action of acid on the process of sensitisation a culture of organisms was suspended in saline with HCl present in concentration equal to N/100 and sensitised with specific agglutinating serum—titre 1/8000—in concentration of 1/100. The mixture was allowed to stand at room temperature for 48 hours, it was then centrifuged at high speed to deposit the bacteria. The supernatant fluid was drawn off and the deposit washed with distilled water. The material was washed twice in this way and then suspended in distilled water. This suspension was added to a series of tubes containing the following reagents in such concentration that, with the addition of the bacillary suspension, the concentrations shown in the following table were obtained.

TABLE I.

B. paratyphosus β and its Homologous Serum. (HCl N/100.)

Electrolyte	N/20	N/100	N/1000	N/10,000	N/100,000
NaCl	—	—	—	—	—
Na ₂ SO ₄	—	—	—	—	—
BaCl ₂	—	—	—	—	—

Note. In the above table and in all which follow,

+ = Agglutination.

— = No agglutination.

? = Doubtful result.

The tests were done using the macroscopic method, the volume in each tube being 1 c.c. In the experiments with *B. paratyphosus* the readings were made after 2 hours at 37° C., with the meningococcus, after 24 hours at 55° C.

Control experiments with a number of strains of the same and other organisms showed that when sensitisation was carried out with sera of much lower titre—1/2000—in absence of acid the organisms agglutinated readily in all cases at a concentration of NaCl equal to N/100. The converse of the experiment with NaOH gave a similar result.

If the reaction of the menstruum then be too acid or too alkaline the union of antigen with antibody is inhibited, and no complex being formed, the system is not susceptible to the flocculating action of electrolytes.

Certain colloidal complexes appear to be more susceptible than others to this disturbing influence, and on this account great care should be taken to prevent the introduction of technical errors.

(b) Influence of the electrolytes of the menstruum.

There is some evidence too (unpublished investigations on biological lysis of red blood cells deprived of their electrolytes by prolonged washing in isotonic sugar solutions) that the formation of an antibody-antigen "couple" is also conditioned by the presence of dissolved salts in the menstruum and that the result obtained depends largely on the nature of the electrolytes in the fluids employed for suspending the reagents. In these experiments it is of course essential that all the fluids used be isotonic with the red blood cells. The "inactive" diluents employed by me in these experiments are solutions of glucose or saccharose.

The work of Pauli¹ on the relation which the concentration of electrolytes bears to the coagulation temperature of protein points in the same direction, for it will be seen from the second section of the present communication that the behaviour of sensitised organisms—i.e. antibody antigen "couple"—strongly recalls the reaction of denaturated proteins, and Pauli's investigations indicate that the process of denaturation is also to some extent conditioned by the presence of salts. It is interesting to note that Pauli's work calls attention to the fact that the reaction between salt and protein in this connection is probably of the nature of a surface condensation.

(c) Influence of the presumably inactive constituents of the antigen and antibody colloids upon the process of sensitisation.

It must be clearly understood that the active substance of (or the peculiarly active physical state of) the serum, or of the organismal colloids, that leads to the initial sensitisation in the process of agglutination, does not necessarily include all the constituents of the serum or of the organism.

The (presumably) inactive substances may be present in such quantity or in such a physical state that they protect the united antibody-antigen complex from the flocculating action of electrolytes. I conceive of certain examples of the "negative phase phenomenon" in agglutination as being due to such a mechanism. The following is an example of such a negative phase reaction with the serum of an animal (rabbit) on being immunised with meningococcus, Type III.

¹ *Zeitschr. Chem. Ind. Koll.* 1908, III. 2.

TABLE II.

Serum of Rabbit "U" in course of Immunisation.

	1/50	1/100	1/200	1/400	1/800
Coccus used for immunisation	-	-	+	+	+

It is to be noted that after being stored for some time this particular serum no longer exhibited a negative phase.

(d) *The quantitative relationship between antigen and antibody in the process of agglutination.*

The quantitative relationship between antigen and antibody in the process of agglutination is of considerable moment, in view of the fact that upon this directly depends the validity of the absorption of agglutinin test in its application to bacteriological research.

Mainly owing to the work of Eisenberg and Volk, this relationship is now known to obey the same laws as those governing the phenomenon of adsorption or surface condensation.

When a foreign substance is introduced into a two phase-colloid-"solution" it tends according to its physico-chemical attributes to distribute itself throughout the system in a peculiar way. Often it will be found to condense itself on the surface of the disperse phase.

For example, if gum arabic be introduced into a tube containing suspended particles of finely divided barium sulphate in water (such a suspension of barium sulphate may be regarded as a suspensoid colloid admittedly of large particle, dispersed in the water) the gum condenses on the surface of the particles of barium sulphate and a complex of barium sulphate plus gum is formed. It may be noted in this instance that the gum arabic acts as a protective colloid and inhibits the sedimentation of the barium sulphate due to gravity.

The amount of foreign substance which a disperse phase can adsorb depends, *caeteris paribus*, upon the extent of the surface which it presents for the foreign substance to condense upon.

The law which governs this process is of wide application in physical chemistry and is applicable to the adsorption of hydrogen and other gases by carbon at different pressures and the adsorption of dissolved substances from various strengths of their aqueous solutions by the same reagent. The law holds good for solutions of any substance in a variety of solvents.

The amount of gas adsorbed, or the quantity of solute removed from a solution by carbon, does not increase either in proportion to the

pressure under which the reaction is carried out, in the former instance, or to the concentration of the dissolved substance in solution in the latter. The amount of gas, or material adsorbed from solution, increases much more slowly in proportion than the increase of pressure exerted, or the heightening of concentration of solute in the fluid.

In the agglutination reaction we introduce serum—a foreign substance—into a two phase system consisting of bacteria dispersed in saline. Such suspensions of bacteria have properties akin to those of non-rigid or emulsoid colloid solutions.

In many respects they recall solutions of fresh proteins which are flocculated like these by high concentrations of ammonium or magnesium sulphate. Like the proteins too, certain suspensions are flocculated by one concentration of those reagents while similar suspensions of other micro-organisms require higher concentrations of these salts to bring this about.

The foreign substance—serum—distributes itself between the saline and the bacteria and if a sufficiently large bacterial surface *of the requisite character* be available, the whole of the serum will be condensed thereon. If the surface be not sufficiently extended, a certain amount of serum will remain unattached and will be demonstrable in the fluid after removal of the antibody-antigen complex by centrifuging.

The following table from Eisenberg and Volk¹ calls attention to the adherence of this reaction to the law of adsorption as the figures of column II are in remarkable agreement with those in column III, the former of which indicate observed results while the latter give the results as calculated on the general formula of adsorption.

The figures of column I represent the amount of agglutinating serum, estimated in arbitrary units, exposed to adsorption by a given (arbitrary

TABLE III.

Column I	Column II	Column III	Column IV
Units of Agglutinin exposed to Adsorption	Units <i>observed</i> after Adsorption	Units which ought to be demonstrable after Adsorption <i>calculated</i> on "The Adsorption Formula"	Units adsorbed (<i>observed</i>) expressed a percentage of that exposed to Adsorption
2	0	0.02	100 %
20	0	0.7	100 %
40	0	2.1	100 %
200	20	19.7	90 %
400	60	52.9	85 %
2000	500	478	75 %
10000	3500	3890	65 %
20000	9000	9160	55 %

¹ *Zeitschr. f. Hyg.* 1902, XL, 155.

but constant) quantity of emulsion of the homologous organism. Those of column II indicate the potency of the demonstrable remaining agglutinin observed in the fluid after the serum and organisms have interacted. The potency of the agglutinin is expressed in terms of the same unitage as in the figures of column I. Column III indicates the potency of agglutinin which should be present after the reaction if the union of antigen and antibody were strictly amenable to the law of adsorption. Column IV indicates the quantity of agglutinin adsorbed, expressed as percentages of the amount exposed to the adsorbing action of the organismal suspension.

The above results are so striking that they scarcely call for comment, but the importance of the findings cannot be exaggerated. It is obvious that the absorption of agglutinin test is limited in its application, and that extreme care must be exercised in its technique if reliable results are to be obtained.

Attention is here called to the qualification noted in the previous paragraph, viz. that the condensing surface must be of the requisite character, that is that the suspension must be homologous with the serum to be adsorbed.

It is this which introduces that factor in the phenomena of immunology—specificity—concerning which we remain in complete ignorance.

Until the conditions which determine the specificity of serological tests are known, all the so-called immunity reactions must remain fundamentally empirical, so that at present their value as an aid to the further investigation of bacteriological problems can only be gauged by the results obtained.

I might also call attention here to the fact that in addition to the specific adsorption of an antibody to its homologous antigen, there may sometimes be some non-specific condensation of antibody upon a heterologous antigen or other disperse phase suspended in the menstruum to which the serum is added. It follows that in carrying out the absorption of agglutinin test one must bear in mind the possibility of thus introducing avoidable error; the test must be performed under such conditions that

- (a) the serum is sufficiently dilute,
- (b) that the emulsion of organisms is sufficiently dense to bring about complete absorption of specific antibodies, but
- (c) that the emulsion is not too dense lest it introduce error owing to non-specific condensation of the serum.

The import of these points will again be noted in considering the

practical application of the absorption of agglutinin test, for it will be seen from the later sections of the present communication that it is necessary to elaborate a special technique for every variety of organism or rather for every group of organisms that one proposes to examine.

(e) *Analogies between the union of antibody with antigen and certain experiments of colloidal chemistry.*

Gengou¹ in an illuminating article on the subject of molecular adhesion calls attention to certain important points. He shows, for example, that the apparently different and opposed phenomena of dissociation and agglutination may be due to one and the same mechanism. Those colloids which are in themselves stable bring about dissociation of material suspended in fluids, while unstable colloids added to the same suspension may bring about its flocculation. This point will be further considered in the second part of this article, which deals with the purely physical factor of agglutination—i.e. precipitation.

One point of great import having its counterpart in the phenomenon of agglutination is, that when a colloid is adsorbed to a suspension of, for example, barium sulphate, there results a barium sulphate colloid complex. If the fluid containing such a complex be centrifuged, it is found that the deposit obtained consists of the whole complex and not of either constituent alone.

The supernatant fluid is inert and will no longer bring about either dispersion or flocculation, as the case may be, of further quantities of barium sulphate suspension that may subsequently be added to it, provided that the quantity of adsorbable colloid added in the first instance is not too large. The law governing adsorption in general is applicable to these complexes.

An especially interesting point may here be noted, namely, that the complex of a colloid adsorbed to a suspension may have a very different effect upon that suspension than does the colloid alone.

Of this phenomenon Gengou also cites an excellent example: gum arabic added to suspensions of barium sulphate markedly stabilises these and renders them relatively insusceptible to gravity. On the contrary, the "BaSO₄-gum complex," obtained by centrifuging such a mixture, brings about very rapid flocculation of further suspensions of barium sulphate in water subsequently added to it. In the first experiment, sedimentation of the barium is inhibited owing to the particles of that substance being protected one from another by a

¹ *Arch. Intern. Phys.* 1908, vii. fasc. 1 and 2.

coating of gum and, as the gum shows no tendency to flocculate, the suspension is stable. In the second experiment, on the contrary, each droplet of gum with its nucleus of barium sulphate forms a particle on the surface of which the barium sulphate which is further added will be adsorbed and condensed.

This second quota of barium sulphate is however unprotected and is therefore liable to flocculate, which it will do all the more rapidly because the particles of the system are much larger and heavier than before, and do not now show the mutual repulsion for one another exhibited by the droplet of gum.

I call attention to these points as they serve to illustrate how apparently paradoxical may be the interaction of relatively simple colloidal systems with one another, and to indicate the paramount necessity for special care being observed when carrying out tests in which the interacting systems appear to be almost infinitely complex as is the case in the immunity reactions.

(f) Influence of the physical state of the reacting systems upon the union of antibody with antigen.

In Part II of the present communication, attention is called to the fact that sensitised organisms—serum-organism “complex”—behave in certain cases as does denaturated protein. It is remarkable that in respect of agglutination, if either of the interacting colloids be denaturated by heat prior to their being mixed together, agglutination may not take place. Indeed, serum exposed to relatively high temperature may even develop inhibitory properties, thus Priestley¹ finds that if agglutinating sera be heated to certain temperatures, which vary according to the serum under investigation,—temperatures between 60° C. and 70° C. for 30 minutes—they may no longer produce agglutination of emulsions of the homologous organism in any dilution; the same serum heated to 75° C. for a similar time may become not only inactive but even inhibiting, and will interfere with the agglutinating power of the (same) unheated serum.

If it be exposed to a still higher temperature it not only fails to produce agglutination, but, further, it loses this inhibitory property.

The same is true to some extent of bacteria. Thus Eisenberg and Volk² find that bacteria heated above 65° C. may no longer agglutinate in presence of immune serum. This seems to be true of most organisms; it has been my experience with representatives of the colon-typhoid

¹ *Journ. Hyg.* 1917, xv. 500.

² *Zeitschr. f. Hyg.* 1902, xl.

group of bacilli, meningococci and *B. tetani* when heated to 75°–80° C. for 30 minutes.

I conceive of this alteration in agglutinability as depending upon an alteration in the physical state of the reagents. Its import cannot be overrated for it implies that there are different optimum conditions for the demonstration of each type of reaction between antibody and antigen, so that here again the essentially empirical nature of the agglutination test is emphasised until at least these optimum conditions have been determined in respect of the particular complex that is under investigation.

What precisely happens on "overheating" an antibody or an antigen is difficult to say and in the first instance—overheating of the serum—the problem appears incapable of solution by simple experiment. Michaelis, Eisenberg and Volk, and Bail, have, however, demonstrated that it is the process of flocculation, and not that of the union of antigen with antibody which is thus inhibited.

In the latter case—"overheating" of the antigen—it seems that the modification of the antigen is such that the complex which it forms with the serum is not susceptible to flocculation by electrolytes. That the antibodies of the serum unite with the organisms is shown by the fact that they are removed by absorption with emulsions of "overheated" bacteria.

The following experiment illustrates this phenomenon in the case of the meningococcus.

An emulsion of meningococcus (a Type III coccus was used) standardised to contain four thousand million cocci per c.c., was divided into two portions, A and B.

A was exposed to a temperature of 65° C. for 30 minutes and B to a temperature of 80° C. for the same time.

Each was then used for saturating a specimen of Type III serum following the standard technique.

The following day agglutination and saturation experiments were set up with A and B, the following results being obtained:

	Unsaturated serum				Saturated serum. Coccus added was heated to 65° C. for 30 m.			
	1/100	1/200	1/300	1/400	1/100	1/200	1/300	1/400
Coccus heated for 30 m. to 65° C	+	+	+	+	—	—	—	—
Coccus heated for 30 m. to 80° C.	+	—	—	—	—	—	—	—

Note. The temperature to which the organisms must be exposed, and the time during which they are exposed thereto, varies considerably. Some organisms are more susceptible than are others to the influence of heat. This probably depends on the reaction of, and the electrolytes present in, the menstrium. Pauli's work on the coagulation of the proteins would lead one to expect such variation.

The complexity of the whole mechanism is further enhanced by the fact that agglutinin so-called is not a definable substance. All organisms contain—in terms of the nomenclature used by Ehrlich and his followers—more than one agglutinin-producing antigen, as is shown by Joos¹. This author shows that in the case of *B. typhosus* one of these or a group of these is thermostable and another, or others, thermolabile. Even more important than the finding of Joos are those of Scheller² that heated bacteria may absorb agglutinins from the sera with more avidity than do emulsions of the same organisms when they have not been heated.

The influence, then, which previous heating exercises upon organisms that are to be agglutinated is not unimportant. Heating to certain temperatures may enhance both the power which a given suspension of organisms has of combining with antibodies, and the flocculability of the complexes which the organisms form with these, while heating to 80° C. although it does not necessarily inhibit the formation of a "complex" does inhibit flocculation.

The conditions under which such heating, before exposure of the suspension to the agglutinating serum is carried out, are important. A saline suspension heated to 65° C. is agglutinable in the case of most organisms, but a similar suspension heated to 65° C. in saline containing 0.5 per cent. pure phenol may not flocculate on exposure to the same serum.

The assumption that there is a multiplicity of agglutinogenic antigens in one organism as conceived by Joos, renders the whole subject of agglutination unnecessarily complex. If it be true, it is not improbable that the optimum conditions for the flocculation of one particular antigen-antibody complex will differ from the optimum conditions for the flocculation of another, although the two antibodies are each constituents of the same serum and the two antigens constituents of the same organism.

In another connection the (assumed) "multiplicity" of so-called agglutinin-producing antigens is of importance, for the more specific response on the part of an animal to inoculation with emulsions of certain organisms occurs more readily than the less specific or group response. There is really another explanation of this which is dealt with in Part II.

This is particularly true of meningococcus and is almost equally

¹ *Centrabl. f. Bakteriöl.* 1903, xxxiii.

² *Centrabl. f. Bakteriöl., Orig.*, 1904, xxxvi. pp. 427, 694; 1905, xxxviii. p. 100.

true of *B. tetani*. For this reason in working with these organisms I invariably employ only the serum of animals whose course of immunisation has not been prolonged beyond ten days.

A similar fact, and one of considerable importance, particularly in the study of the pathogenic anaerobes, is, that if an animal be immunised with mixed cultures of certain organisms, the antibodies for one of the organisms comprised in the mixture may be demonstrable in the blood serum of the animal some days before those corresponding to the others are manifest.

It has so far been my experience—although this is admittedly limited—that the response of the animal to the more pathogenic constituent of the mixture occurs earlier and is more easily demonstrable than is the response to the less pathogenic constituent.

II. THE SECOND PHASE OF AGGLUTINATION.

The second phase of the agglutination test, that of demonstrating the formation of a complex by its flocculation, is relatively simple compared with the first phase. Experiments conducted with a view to its elucidation in the case of organisms of the colon-typhoid group show the mechanism of flocculation of sensitised bacteria in this group to be similar to that concerned in the aggregation of suspensions to certain denaturated proteins.

It must be remembered in this connection that different proteins react differently in the presence of various reagents, as is shown by Chick and Martin¹. These authors demonstrate that the aggregation of particles of denaturated egg-white is conditioned by the presence of electrolytes and by the range of hydrogen ion concentration over which the reaction is extended.

The same is true of the aggregation of particles of denaturated serum-protein but the figures applicable to egg-white are not applicable to serum-protein, etc.

In the case of serum-protein, the flocculation of the particles occurs when electrolytes are present in low concentration, and if these be raised to certain concentrations which bring about aggregation of egg-white, no aggregation of serum-protein occurs.

In the same way it is highly probable that certain antibody-antigen complexes are much more susceptible to the flocculating, or to the dispersing, power of electrolytes than are other similar complexes—

¹ *Journ. Physiol.* 1912-1913, XLV, 261 and 295.

that is "similar" only in that they consist of organisms, which have adsorbed their homologous antibodies.

Considering, then, the second phase of the reaction, one may ask the question, Why is it that a suspension of organisms remains more or less stable until the organisms are sensitised? when the organisms are sensitised, What is the mechanism which determines their clumping together in masses sufficiently large to render them susceptible to gravity?

As Liefman has shown¹, unsensitised organisms are flocculated only by the process of salting out by means of highly concentrated solutions of magnesium sulphate and ammonium sulphate and therein resemble natural proteins.

Their resemblance to fresh proteins is all the more marked in that some species of organisms are precipitated by certain concentrations of these reagents, while others require stronger solutions to bring this about.

Sensitised organisms—serum-organism complex—on the contrary, behave like denaturated proteins and, like rigid colloids, are susceptible to flocculation by low concentrations of salts.

The unsensitised organisms in a suspension are repelled by and themselves repel, those adjacent to them by virtue of their carrying a surface electric charge according to the Lippman-Helmholtz hypothesis. The conditions which bring about the neutralisation of this charge will determine the flocculation of the suspension.

The charge carried by protein particles in suspension depends in kind and in degree upon

- (1) the reaction of the menstruum in which they are suspended,
- (2) the electrolytes present.

In enquiring into the second phase of agglutination therefore the relation which these two factors bear to one another must be considered and the relation which each, or both, bear to the degree of sensitisation to which the organisms have been subjected. Furthermore the influence which other physical conditions may have upon the process must not be lost sight of.

(a) *Influence of the reaction of the suspending fluid, in which the interacting bodies are dispersed, upon the process of flocculation, and influence of the valency of the electrolytes upon the process in presence of acid and alkali.*

Considering these questions *seriatim*, it is found that the reaction of the suspending fluid exerts a marked influence on the phenomenon under

¹ *Centralbl. f. Bakteriol., Referate*, 1913, LXXV. 14.

consideration. This would naturally be expected in view of the fact that the surface charge carried by particles of protein—organisms may be regarded as large protein particles—in suspension depends for its sign upon the acidity or alkalinity of the fluid. When the menstruum is acid the particles carry a positive charge and when alkaline, a negative charge.

The following tables illustrate experiments designed with a view to showing the influence of acid and alkali upon the process of agglutination of sensitised bacteria.

METHOD. A 24 hours' agar culture of *B. paratyphosus* β is washed off in saline and exposed at 22° C. or 37° C. for 24 hours to the action of 1/1000 anti-paratyphosus β serum of titre 1/8000.

The supernatant fluid is pipetted off and the deposit washed by centrifuging at least twice in distilled water. The deposit is then suspended in distilled water, filtered to remove any gross particles that may remain and exposed to the action of NaOH N/100 in varying concentration in presence of a number of electrolytes, each of which was, when all the reagents were mixed, equimolecular with 0.9 per cent. NaCl. The following table shows the readings of this experiment after 2 hours at 37° C.

TABLE V.

Bacillus paratyphosus and Homologous Serum.

Complex—constant. Electrolyte—constant. Hydroxide Ion—variant.
2 hours at 37° C.

Electrolyte	NO NaOH Control	1 c.c.	0.9 c.c.	0.8 c.c.	0.7 c.c.	0.6 c.c.	0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.
NaCl	+	—	—	—	—	—	—	—	—	—	+
K ₂ SO ₄	+	—	—	—	—	—	—	—	—	—	+
KI	+	—	—	—	—	—	—	—	—	—	—
NaHCO ₃	+	+	+	+	+	+	+	+	+	+	+
Na ₂ HPO ₄	+	+	+	+	+	+	+	+	+	+	+

This experiment shows that the hydroxide ion interferes with the process of flocculation and that a relatively small concentration of that ion has a marked inhibitory effect. The results with NaHCO₃ and NaHPO₄ show that if replaceable hydrogen be present in the electrolyte this inhibitory effect is negatived.

The converse of this experiment is shown in the following table. The organisms were sensitised with 1/100 serum, then washed twice as before and exposed to the action of varying proportions of N/50 HCl in presence of the electrolytes named.

TABLE VI.

Bacillus paratyphosus and Homologous Serum.

Electrolyte N/20	Complex—constant.		Electrolyte—constant.				Hydrogen Ion—variant.				
	2 hours at 37° C.										
	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	
NaCl	—	—	—	—	—	—	—	—	—	—	
Na ₂ SO ₄	—	—	—	—	—	—	—	—	+	+	
NaCit (Neutral)	+	+	+	+	+	+	+	+	+	+	
BaCl ₂	—	—	—	—	—	—	—	—	—	—	

Note. The citrate result is "falsified" in respect of the influence of valency owing to the replaceable Na of the salt.

It may be noted that the above results indicate that the inhibitory effect of hydron is more marked in the presence of monovalent anions than in the presence of divalent anions. The reason for this will be considered later.

I have purposely omitted from this section of the work a detailed consideration of the influence of polyvalent kations or anions upon the process of flocculation, as these are somewhat difficult to investigate. The polyvalent kations have much the same influence upon unsensitised organisms as they do upon sensitised organisms, owing probably to the formation of metal hydroxides in the system in the form of precipitable gels. The polyvalent anions also present difficulties owing to their dissociation; thus, using citrates, if a neutral solution of the salt be employed the following difficulty arises, the effect of acid cannot be readily estimated owing to the presence of available hydroxide which will neutralise the acid, while the effect of alkali is also masked owing to the presence of replaceable hydrogen groups.

The mechanism whereby electrolytes bring about the precipitation of sensitised organisms is explicable on the basis that they neutralise the surface charge carried by the particles. It follows therefore that the precipitating ion in the case of acid suspensions of protein is the anion, while in that of alkaline suspensions, it is the kation. It is interesting to note that Burton¹ found that the addition of aluminium ion to hydro-sols of gold or silver in certain concentrations, leads to the precipitation of the metal from its sol; lower concentrations of the Al ion did not have this effect, and the particles of the sol in the case of the metals under consideration (which bear a negative charge) continued to move in the same direction in an electric field as before *but at a diminished rate*.

¹ Univ. Toronto Stud. Phys. Lab. 36.

If concentrations of Al ion, greater than that required to bring about the precipitation of the sol, were employed, the particles remained dispersed, but their direction of movement in the electric field was reversed, showing that they had now become positively charged.

In the case of the sopper sols, which carry a positive charge, exactly the opposite was noted: polyvalent anions in this case lead to precipitation, and ultimately if added in sufficient concentration to dispersion, accompanied by a reversal of surface charge as evidenced by reversal of direction of movement in the electric field.

The relative precipitating value of uni-, di- and tri-valent kations in respect of negatively charged colloids and of uni-, di- and tri-valent anions in respect of those bearing a positive charge may be expressed according to Linder and Picton¹ by the formula: $1 : x : x^2$.

We have evidence that, in the case of sensitised bacilli of the colon typhoid group at least, the precipitating value of salts obeys in general the same rules in respect of valency, although the actual figures applicable to the precipitation of colloidal As_2S_3 (the reagent used by Linder and Picton) by electrolytes are not applicable to the flocculation of serum-organism complexes. These facts show that in acid suspension or in alkaline suspension, sensitised bacteria behave as do denaturated protein particles, and the results obtained agree in principle with the findings of Hardy² and of Chick and Martin³.

I here recall the fact that these authors show that the precipitation of denaturated egg-white is determined by somewhat different physical conditions from those that bring about precipitation of denaturated serum-protein. It follows therefore that the optimum conditions for the flocculation of each type of antigen-antibody complex would have to be defined before the most specific agglutination results could be obtained. Here again the essentially empirical character of the agglutination test, as ordinarily performed, is manifest.

(b) *The relation which exists between the degree of sensitisation and the precipitating value of the electrolytes present in the menstruum.*

This point is of considerable importance especially if a proper appreciation of the absorption of agglutinin test is to be obtained. The following examples indicate that this relationship is not a simple one, and they serve to call attention to its important bearing upon the question under consideration.

¹ *Journ. Chem. Soc.* 1895, LXVI. and LXVII.

² *Journ. Physiol.* XXIV. 170 and *Proc. Roy. Soc.* 1900, LXVI. 101.

³ *Journ. Physiol.* XLV. 261.

If emulsions of *B. Aertrycke* and *B. paratyphosus* β be exposed to a serum specific to either, it not infrequently happens that both organisms are equally well agglutinated. One may describe such a finding thus: that both *B. Aertrycke* and *B. paratyphosus* β form with e.g. anti-paratyphosus β serum, complexes which are flocculable by 0.9 per cent. NaCl.

It does not necessarily follow, however, that these two complexes are identical, and if one can show that the complexes differ from one another, the validity of the absorption test, in its application to the identification of micro-organisms, is much enhanced. In effect the proposition is: Does *B. paratyphosus* β form exactly similar (that is, similar from the standpoint of flocculation) complexes with its homologous serum as does *B. Aertrycke* with the same, and therefore heterologous?

A priori, one might hazard a guess that this query is to be answered in the negative; for, were it answered otherwise, the absorption of agglutinin test would be inexplicable. By varying both the dilution of a serum and the concentration of electrolytes in which an agglutination is carried out, it can be shown that a whole series of complexes, varying in their susceptibility to flocculation, may be formed when an organism is exposed to the action of its homologous serum. The following table illustrates an experiment of this kind in which both serum and electrolyte are variants while the organismal suspension is a constant.

TABLE VII.

Electrolyte = NaCl					Dilutions of Serum
N/20	N/40	N/100	N/200	N/400	
+	+	+	+	+	1/100
+	+	+	+	+	1/500
+	+	+	+	?	1/1000
+	+	+	?	-	1/2000
+	+	?	-	-	1/4000
-	-	-	-	-	1/8000

Electrolyte = BaCl ₂					Dilutions of Serum
N/400	N/500	N/666	N/1000	N/2000	
+	+	+	+	+	1/100
+	+	+	+	?	1/500
+	+	+	-	-	1/1000
+	+	-	-	-	1/2000
+	-	-	-	-	1/4000
-	-	-	-	-	1/8000

This experiment shows that an organism can form, along with its own antibody, a variety of complexes differing *inter se* in their suscep-

bility to flocculation. These complexes cannot be regarded as different in kind, but only in degree. Is it not highly probable then that the complex formed by union of an organism with an heterologous antibody would differ markedly from that obtained by interaction with homologous antibody?

Light could be thrown on this problem by setting up an experiment in which the suspension of organisms and the serum remain constant, the only variant being the concentration of electrolytes. The following experiment illustrates this.

METHOD. Equal volumes of standard suspensions of *B. Aertrycke* and *B. paratyphosus* β were each sensitised in presence of anti-Aertrycke and anti-paratyphosus β sera. The agglutinated bacilli were washed twice in distilled water, shaken and filtered through paper to produce an homogeneous suspension which was then "dispersed" in distilled water. Thereafter each of the emulsions so obtained was exposed to the influence of varying concentrations of NaCl and of BaCl₂. The following results were obtained.

TABLE VIII.

1 hour at 37° C.

Serum used			Organism	Concentrations of Electrolyte, NaCl					
				N/20	N/40	N/80	N/100	N/200	N/400
Anti-Aertrycke serum	B. Aertrycke	+	+	+	+	-	-
Titre=1/2000	B. paratyph. β	?	-	-	-	-	-
Anti-paratyphosus β serum	B. Aertrycke	+	+	+	-	-	-
Titre=1/8000	B. paratyph. β	+	+	+	+	+	+

The results obtained with BaCl₂ are not shown in the above table. They fully corroborate those obtained with NaCl.

Note. When a simple agglutination was set up in saline with the above organisms in presence of homologous and heterologous sera, it was found that the anti-Aertrycke serum agglutinated *B. Aertrycke* and *B. paratyphosus* β equally well and *vice versa*.

The result indicates, I think, that serum and electrolyte being constant, the physical properties of what might be termed a "co-complex" differ from those of what might be called a "specific complex," the latter being more susceptible to the flocculating action of electrolytes. This result also bears a striking resemblance to Pauli's investigations noted in Section I of the influence which salts have upon the heat coagulation of protein, for it appears that the salts in Pauli's experiments may have played a double rôle

- (1) by influencing the process of denaturation and
- (2) by determining the occurrence of actual flocculation.

The relation which the valency of the precipitating ion of the electrolyte bears to the titre of the serum here calls for attention. If a number of electrolytes equimolecular with NaCl be used in place of that salt, it is found that none of them increase the titre of the agglutinating serum as is shown by the following table illustrating such an experiment:

TABLE IX.

Electrolyte	Dilutions of serum						
	1/100	1/500	1/1000	1/2000	1/4000	1/8000	1/10,000
NaCl	+	+	+	+	+	+	-
NaF	+	+	+	+	+	+	-
Na ₂ SO ₄	+	+	+	+	+	+	-
K ₂ SO ₄	+	+	+	+	+	+	-
Na ₂ CO ₃	-	-	-	-	-	-	-
Na ₂ HPO ₄	+	+	+	+	+	+	-
BaCl ₂	+	+	+	+	+	+	-
NaCit (Neutral)	+	+	+	+	+	+	-
Aq. Dest.	+	-	-	-	-	-	-

Note. The positive result with 1/100 dilution of serum is probably due to the salts which the serum contains.

On comparing this result with that indicated in Table VII one sees that as one approaches that dilution of serum which might be called its "threshold value" the electrolyte has to be added in greatly increasing concentration, in order to bring about aggregation of the bacteria.

Thus in Table VII it is seen that BaCl₂ brings about flocculation in concentration—N/400 in presence of 1/4000 serum—while an increase in concentration of the same salt to N/6.5 does not show marked increase in the titre, which is apparently the same as that with NaCl 0.9 per cent.

(c) *Effect of other physical factors upon the second phase of agglutination.*

It might be expected that the surface tension—intrinsic pressure—and viscosity—internal friction—of the menstruum, would influence the process of flocculation because of the important rôle which these play in determining the disposition of particles in suspension. It has been found, however, that, within fairly wide limits, provided the electrostatic conditions of the particles in suspension do not show alterations concomitant with variation of these, they in themselves have but little effect in enhancing or inhibiting the aggregation of sensitised bacteria.

One point which is so obvious that it may easily be forgotten, is drawn attention to by Gengou: that if the particles deprived of their

surface charge are to come together to form flocculi, they must not be too far dispersed from one another in the suspension, for, under such circumstances their coalescence will be delayed and the initial lag in this phase of the reaction may be lengthened beyond the time arbitrarily allowed in the laboratory for the demonstration of agglutination.

Admittedly this must occur but infrequently. Nevertheless, attention is called to it because it substantiates the plea that to be of value for comparison the agglutination test must be carried out under standard conditions. One of the most easily standardised factors in the process is the bacterial content of the emulsion employed.

The temperature to which the reagents are exposed before or during the test is also of moment, for variation of this condition may well affect both phase I and phase II of the reaction.

I have already discussed how it may influence the union of antigen and antibody. Its influence on the process of flocculation is even more striking, for it may affect this in two distinct ways:

(1) by producing a continuous movement of the interacting bodies in the suspension,

(2) by encouraging or inhibiting, owing to its altering the physical state of certain complexes, their precipitation by electrolytes.

Considering these factors in more detail the following points appear:

(1) It is known that gentle shaking of an antibody-antigen complex suspended in saline will hasten in a remarkable manner the process of flocculation. This hastening of the process is probably due to the fact that shaking brings the particles of the suspension closer together and makes one particle roll over the other. As these particles are no longer mutually repellent, they tend to coalesce and having once coalesced remain together unless violently dispersed by rough handling.

If the reaction be carried out at 55° C. I have observed that the convection currents in the tubes are more active than if the test be made at 37° C. These convection currents have exactly the same effect as gentle shaking of the tubes and are not too active at this temperature to break up the flocculi. The higher temperature therefore is, *ceteris paribus*, a more satisfactory one at which to perform the test than is body heat; provided always of course that the possibility of introducing an experimental error due to this elevation of temperature is borne in mind and such error eliminated before incubation at the higher temperature is adopted as a routine procedure.

(2) The mechanism whereby the higher temperature enhances flocculation depends on the fact that certain proteins are more easily

flocculated after exposure to an increase in temperature varying according to the particular protein under examination: they are in fact altered somehow in respect of their physical properties.

Gengou, in the article already quoted, gives a striking example of this. If unheated serum be mixed with a suspension of BaSO_4 , the serum stabilises the suspension; but if the serum be previously heated to 65°C . it induces rapid flocculation of suspensions of the same substance. In the former instance, the serum, adhering to the BaSO_4 , and itself showing no tendency to flocculate, protects the BaSO_4 , so increasing the stability of the suspension. In the latter instance, on the contrary, the serum does tend to flocculate of its own accord and adhering to the BaSO_4 , which is a fairly heavy substance, remaining in suspension in water only for a limited time, and only if it be sufficiently finely divided, the union of the already flocculable protein to the heavy barium suspension will result in aggregation and sedimentation taking place with great rapidity.

One would naturally expect then that as proteins are rendered more susceptible to flocculation by elevation of temperature, that the formation of certain complexes, while not demonstrable at 37°C ., might well be demonstrable at, for example, 55°C . This is particularly likely to be the case for the process of sensitisation, i.e. the formation of a complex, as we have seen, is akin to the process of denaturation.

There is an obvious criticism to the employment of the higher temperature for agglutination—Does the reaction remain specific under the new conditions brought about by the change from 37°C . to, e.g. 55°C .? The answer to such criticism is to be sought by experiment and in experience.

If a sufficient number of organisms be investigated under the new conditions, if a sufficiency of controls be included in the experiments—always especially bearing in mind the employment as a control of a normal serum of the same species of animal as that used for preparing the agglutinating serum—if the results obtained are corroborated by the absorption of agglutinin test, the new conditions may be found to be valid in that they yield specific results.

From this again, it can be seen that the test is empirical, for until we know the optimum conditions for the demonstration of agglutination in respect of these factors, the value of the agglutination and the absorption of agglutinin tests, for the identification of micro-organisms, can only be established on results obtained.

III. PRACTICAL CONSIDERATIONS AND DISCUSSION.

The points raised in Sections I and II of this communication indicate that the whole process of agglutination depends upon a number of finely balanced physico-chemical reactions. The formation of a flocculable "complex," and its sedimentation, are both governed by physical forces, and it is apparent that the fine balancing of these forces may in some instances be of paramount import if consistent results are to be obtained.

In carrying out the agglutination test and the absorption of agglutinin reaction, it is essential that those factors which are susceptible of standardisation be standardised; especially in view of the complexity of certain of the reagents employed.

(1) While serum cannot be standardised satisfactorily, as its activity can only be estimated in terms of purely arbitrary units, I think that it is advisable to employ only the serum of animals whose course of immunisation has been of short duration.

(2) To use only a serum which is found to react consistently with several representative strains of each serological type of organism which it is proposed to investigate, and

(3) To elaborate a standard routine technique and scrupulously to adhere thereto throughout each series of experiments which are to be compared one with another. The standards adopted for the examination of one group of micro-organisms may differ considerably from those adopted for the study of another group, e.g. typhoid technique differs somewhat from that used for tetanus.

The standardisation of technique involves:

(a) The employment of sera of approximately equal titre in all experiments dealing with allied organisms. The titre selected for one species would not necessarily be that selected for another, but in respect of all members of one group the titration of the serum to a definite point should be rigidly adhered to.

(b) The use of standardised suspensions of organisms of approximately definite bacterial content and always prepared in exactly the same way, both for the agglutination test and for the absorption of agglutinin test.

The need for the exercise of such care in the preparation of the suspensions is emphasised by the findings of Scheller¹ who shows that higher agglutination titres are obtained with *B. typhosus* previously heated to 65° C. than are obtained with the same organism unheated.

¹ *Centrabl. f. Bakteriöl. Orig.*, 1904, xxxvi. pp. 427, 694 and 1905, xxxviii. p. 100

Further, these heated bacilli are more avid of antibodies than are the unheated organisms.

(c) Saline or other electrolyte employed should be made only with distilled water and, as it is easily prepared, should be made up freshly each day.

(d) The nature of the medium on which the cultures are made, especially the standardisation of the reaction, should be noted, and care should be taken to avoid injuring the medium. It is also advisable to get rid of condensation water before proceeding to wash off the growth. The reason for these precautions is that if the medium be alkaline there is danger that a too alkaline suspension be obtained and so inhibit the reaction. If medium, especially agar, be present in the suspension, it is conceivable that it may act as a protective colloid and so may inhibit both the union of antigen with antibody and the process of flocculation.

(e) I have found that, when performing the absorption of agglutinin test in the examination of meningococci, it is advisable to use freshly prepared sera as these give clearer cut results than do sera which have been in store for some months, even under the best conditions. The reason for the discrepance between freshly made sera and those that have been stored, I am not at present in a position to explain.

(f) If carbolised suspensions are used the strength of the phenol should not be greater than 0.5 per cent. It should only be added after the emulsion is heated—if heated emulsions are employed—and only the purest phenol can be added to suspensions if comparable results are to be obtained.

The results quoted in Sections I and II of this communication show why we are compelled to standardise the reagents and the procedure of the reactions under consideration owing to the errors which are liable to be introduced, were such not done.

If the standards just laid down be adhered to, the results obtained over long periods are found to be comparable with one another and, notwithstanding the apparent complexity of the tests, they are relatively easily carried out and give consistent results after a certain amount of experience has been obtained. Indeed when the necessary experience has been obtained, they are carried out more easily and quickly than are fermentation tests, for these call for extreme care in the isolation of cultures and in the assessment of the results given by growth in the carbohydrate media. Moreover the reactions with these may be much delayed. When a positive reaction is obtained in the case of a fermentation test one must be assured that the result is due to the organism under

examination and not to a contamination. Any one who has been engaged on the study of certain groups of bacteria and notable on a study of the anaerobes, cannot but be deeply impressed with the possibility of error due to this cause in case of inexperienced workers. On the other hand, when a negative result is obtained in the case of a carbohydrate test, one must be satisfied that not only did the inocula live in the sugar medium, but that multiplication took place. These points can only be verified by subculture and re-isolation which occupies valuable time, and the results obtained are worthless, unless the investigator be highly skilled.

It has been my experience that, when my agglutination tests have given unsatisfactory and discrepant results, some detail of my technique has been at fault and a repetition of the test with rigid attention to detail always resulted in consistent findings being obtained.

It will be noted that the question of specificity has not so far been discussed in the present communication, for reasons indicated in the introductory paragraph. I wish to point out, however, that the theory of specificity of serum reactions, which, consciously or unconsciously, dominates nearly all the writings on the subject, is the purely philosophical hypothesis of Ehrlich. This savant visualised organisms as consisting of a number of definite "antigens" certain of which are common to each member of a group of organisms and others specific to individual members (serological types) of the group, in exactly the same way that he visualised each type of antibody as having a peculiar chemical structure. Thus an "agglutinin" was diagrammatised as having a combining chemical group and a flocculating chemical group, and an "amboceptor" as having one group which combined with "antigen" and another with complement. Whenever a new phenomenon was demonstrated in serology, it was only necessary to add another side-chain, or construct a new diagram, and the new reaction was explained.

According to this view, each of the antigens of one organism is assumed to be able to call forth, when that organism is inoculated into an animal, a response specific to itself and the antibodies for each are assumed to be chemically different one from another.

Such an assumption is not by any means necessarily valid, and indeed, if we assume without question its validity, it follows that the experiment described in Section II, subsection (c) (Table No. VII) of this article, would indicate that the complex formed by *B. paratyphosus* β with 1/100 dilution of its homologous serum in presence of NaCl

N/400 might differ not merely in degree but in kind from the complex resulting from the union of the same quantity of the same emulsion with 1/4000 dilution of the same serum in presence of the same electrolyte, or with N/20 NaCl in presence of the lower dilution of the serum.

I conceive, therefore, of group reactions being due possibly, not to closely allied organisms having a definite group antigen common to all the members of the group, but to each member of a group of allied types or related species consisting wholly of specific antigens which however form complexes with heterologous sera more or less easily flocculated in presence of certain concentrations of certain salts, depending upon how close or how remote the relationship may be between the serum and the organism forming the complex.

To conjure up a new antigen common to all those members of a group of organisms which happen to react with one serum, and to extend the process indefinitely as has been done in order to explain why sera are not strictly specific, lays the whole subject of agglutination and other serological tests under suspicion. The same objection may justifiably be made to such reasoning concerning the multiplicity of antigens in one organism as Bordet—rightly I think—advanced in this criticism of the whole side-chain theory of immunity. Bordet says: “Everyone agrees naturally that the numerous antibodies which the study of immunity has brought to our knowledge and which are active on such different elements as bacteria, cells, toxins and the like, should not be considered as identical, inasmuch as they may be distinguished as regards specificity, or, in other words, since they unite with different antigens. But, in addition to this incontestable difference of specificity, Ehrlich has imagined another one which is more far-reaching and which deals with the molecular structure of the antibody. Indeed he classes these antibodies in accordance with their molecular structure into three genera: antitoxins, with a single combining group, agglutinins and precipitins with a single combining group and an additional functional group which brings about agglutination or precipitation; and finally sensitisers which have two combining groups in their molecules, uniting on the one hand with the cell that is affected and on the other side with the alexin (complement) and hence the name of amboceptor.

“In every instance, according to this classification, the phenomena observed are attributed to special properties in the antibody and never to those in the antigen. As a matter of fact, these phenomena should be related not as regards antigen or antibody considered separately, but as regards the complexes which result from their union, and it is

evident that the special properties of the antigen must affect markedly, and perhaps to a preponderating degree, the qualities of such complexes."

It is apparent from this quotation that Bordet's attention is focussed especially upon the antibody. But when the above was written the evidence was not clear that the same criticism could be applied to Ehrlich's conception of antigens. Indeed Bordet apparently agrees to some extent with Ehrlich's view, or at least he does not specifically refute it, that there may be a number of *definite* and therefore chemically different agglutinogenic antigens in one variety of micro-organism. In view, however, of the more recent findings in the study of the physico-chemical aspect of the subject of serology, it is quite logical to apply the same reasoning to the antigen as Bordet applies to the antibody. Further, in view of the results indicated in Section II, subsection (b), of this communication the application of the criticism is even wider than that indicated by Bordet in respect of antibodies, since it can be applied to the discussion of the mechanism of agglutination in the presence of heterologous serum in contrast to that in presence of homologous serum.

Regarded superficially this concept would appear to invalidate the application of the absorption of agglutinin test to the identification of micro-organisms. For, if a complex be formed, the flocculation of that complex should result in removal of antibody from the fluid, as stress has been laid upon the fact that neither organism nor serum is flocculated in the process of agglutination, but that the floccules consist of both serum and organism in some form of physico-chemical combination.

Far from invalidating the test, however, this argument could be used further to establish its value. It is known that some complexes are especially easily flocculated under certain circumstances and that in such cases only a very low concentration of antibody requires to be united with the organism in order to determine the formation of a complex flocculable in presence of e.g. 0.9 per cent. NaCl. The results obtained, performed with suspensions made in saline, indicate therefore that the degree of adsorption of antibody in the case of heterologous mixtures is much less than in that of homologous mixtures and indeed, in most cases, the adsorption which occurs with heterologous mixtures is almost negligible. This really means that phase I of the agglutination test is always specific, while phase II shows specific results only if the physical conditions of the experiment are such as to allow of the specificity of phase I being demonstrated.

IV. RESULTS OBTAINED IN APPLYING THE AGGLUTINATION AND THE ABSORPTION OF AGGLUTININ TEST TO THE INVESTIGATION OF GRAM-NEGATIVE COCCI WHICH PRODUCE PRIMARY MENINGITIS IN MAN.

In view of the fact that the validity of applying the agglutination and absorption of agglutinin tests to the demarcation of the meningococcus group of organisms, and to the differentiation of these *inter se*, may be regarded as debatable. I think it would be of some interest to give a summary of the results obtained in the examination by the methods in question of a consecutive series of this organism obtained from the cerebro-spinal canal.

We may divide those results into those which are quite specific and show that the organism under examination when tested by means of the agglutination method, using the four type sera of Gordon, react with one and with only one of these.

(a) *Results which are absolutely unequivocal in respect of the four type sera.*

Provided that the sera issued by the Central C.S.F. Laboratory for the investigation of epidemic meningitis among troops, were absolutely specific, and if these sera represent all the organisms which might be designated meningococci, every specimen of coccus isolated from the cerebro-spinal fluid in cases of the disease should, when tested with these sera, give a reaction corresponding to one of those symbolised as *a*, *a1*, *a2*, etc. in the following table. No specimen should be met with giving a reaction corresponding to that symbolised as *x*.

It is, however, too much to expect of this, as of any biological method of investigation, that the experimental error will be completely eliminated. It would indeed be surprising if the gram-negative diplococci which produce primary meningitis in man did not show relationship one to another when tested by biological methods.

Of the 356 cocci investigated by the agglutination test in the Central Laboratory, 234 gave absolutely specific results as follows:

- (1) 54 different specimens gave a positive reaction to a dilution of 1/400 with type I serum only.
- (2) 13 reacted with type I serum to a dilution of 1/200.
- (3) 1 reacted with this serum to a dilution of 1/100.
- (4) 80 gave unequivocal results to a dilution of 1/400 with type II serum and reacted with no other serum.
- (5) 39 behaved similarly to a dilution of 1/200 of the same serum.

TABLE X.

"Formula" of Reaction	Dilutions of Type Agglutinating Sera											
	Type I			Type II			Type III			Type IV		
	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400
<i>a</i>	+	+	+	-	-	-	-	-	-	-	-	-
<i>a1</i>	+	+	-	-	-	-	-	-	-	-	-	-
<i>a2</i>	+	-	-	-	-	-	-	-	-	-	-	-
<i>b</i>	-	-	-	+	+	+	-	-	-	-	-	-
<i>b1</i>	-	-	-	+	+	-	-	-	-	-	-	-
<i>b2</i>	-	-	-	+	-	-	-	-	-	-	-	-
<i>c</i>	-	-	-	-	-	-	+	+	+	-	-	-
<i>c1</i>	-	-	-	-	-	-	+	+	-	-	-	-
<i>c2</i>	-	-	-	-	-	-	+	-	-	-	-	-
<i>d</i>	-	-	-	-	-	-	-	-	-	+	+	+
<i>d1</i>	-	-	-	-	-	-	-	-	-	+	+	-
<i>d2</i>	-	-	-	-	-	-	-	-	-	+	-	-
<i>X</i>	-	-	-	-	-	-	-	-	-	-	-	-

The cultures reacting according to formulae *a2*, *b2* and *c2* have been classed as not agglutinating, as, without verifying such a finding by means of the Absorption Test, and thereby definitely placing such cocci, it would be inadvisable to regard them as conforming to any of the four serological types.

(6) 1 coccus reacted in presence of 1/100 dilution of type II serum.

(7) 20 cocci reacted specifically to type III serum to a dilution of 1/400.

(8) 3 reacted similarly, but to a dilution of 1/200.

(9) 16 cocci reacted specifically to type IV serum up to a dilution of 1/400.

(10) 7 gave a specific reaction with this serum to a dilution of 1/200.

That is, 234 of the 348 cocci gave unequivocal type results.

In making this total of the number of cocci which react specifically, I have excluded two strains, No. 3 and No. 6, owing to their poor agglutination.

(11) 8 strains of the organism failed to react in any way with any of the four type sera. Two of these were forwarded to the laboratory not as cultures but as suspensions.

Commenting on these results, it is remarkable, when it is borne in mind that we are dealing with what are admittedly closely-allied organisms, that so high a proportion is susceptible to classification by the test employed.

(b) *Results in which the reactions are not absolutely specific but show a group relationship.*

(1) Examples of what may be called the 1-3 group type and the 2-4 group type.

Most authorities on the meningococcus agree that there are two main types of organism included in the meningococcus group. By employing the absorption of agglutinin test, it has been shown that type I of the French authorities includes a small sub-group which, tested by this method, fails to absorb its antibodies from type I serum.

This small group is designated type III at the Central Laboratory and it will be seen from the results in the previous paragraph that a number of strains of this organism are differentiated by agglutination alone from the other types, including type I, by means of a specific type III serum.

In view of the relationship, we should expect that a number of organisms which react with type I serum would also react to a greater or less degree with type III serum.

In all, 63 specimens of the organisms under consideration react with both type I and type III serum. The following table indicates the degree of reaction obtained with these cocci.

TABLE XI.

"Formula" of Reaction	Dilutions of Type Agglutinating Sera											
	Type I			Type II			Type III			Type IV		
	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400
A	+	+	+	-	-	-	+	-	-	-	-	-
B	+	+	+	-	-	-	+	+	-	-	-	-
C	+	+	-	-	-	-	+	-	-	-	-	-
D	+	+	-	-	-	-	+	+	+	-	-	-
E	+	-	-	-	-	-	+	+	+	-	-	-
F	+	-	-	-	-	-	+	+	-	-	-	-
G	+	+	+	-	-	-	+	+	+	-	-	-

(1) 31 cocci react in a manner corresponding to the formula designated A in the above diagram.

(2) 19 according to formula B.

(3) Three according to formula C.

These may be regarded in all probability as type I cocci.

(4) Three react according to formula D.

(5) Two to formula E.

(6) One to formula F.

(7) Four to formula G.

These organisms were all proved on subsequent examination—even those reacting to formula G—by the absorption of agglutinin test to be identical with either type I or type III cocci.

As in the case of the 1-3 group a similar affinity is noted between groups 2 and 4. Type IV is not recognised by the French authors and like type III in its relation to group I is regarded by some as merely a variant of II. Using the absorption of agglutinin test, type IV can be clearly differentiated from type II. The following table illustrates the reactions obtained with specimens of cocci which show agglutination in presence of type II and type IV serum.

TABLE XII.

"Formula" of Reaction	Dilutions of Type Agglutinating Sera											
	Type I			Type II			Type III			Type IV		
	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400
A	-	-	-	+	+	+	-	-	-	+	-	-
B	-	-	-	+	+	+	-	-	-	+	+	-
C	-	-	-	+	+	-	-	-	-	+	-	-
D	-	-	-	+	-	-	-	-	-	+	+	+
E	-	-	-	+	+	-	-	-	-	+	+	-
F	-	-	-	+	+	+	-	-	-	+	+	+

- (1) 12 cocci react according to formula B.
- (2) Three according to formula C.
- (3) Two react to formula C.
- (4) Three react to formula D.
- (5) One reacts to formula E.
- (6) Seven react to formula F.

In all then 28 specimens give II-IV group reactions, 17 of these are presumably type II cocci, three are presumably type IV and eight are equivocal, reacting equally well with type II and type IV sera.

The total number of organisms giving I-III and II-IV group reactions is therefore 91.

(2) There remain, therefore, 23 cocci which cannot be placed either as specifically type cocci or as cocci showing the common group relationship of I-III or II-IV.

The following table illustrates the reactions of these cocci with the four type sera.

TABLE XIII.

Register No. of Culture	Dilutions of Type Agglutinating Sera											
	Type I			Type II			Type III			Type IV		
	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400
29	+	+	+	+	-	-	-	-	-	-	-	-
42	+	+	+	+	-	-	-	-	-	-	-	-
72	+	+	+	+	-	-	+	-	-	+	-	-
70	+	+	+	+	-	-	+	-	-	+	+	-
174	+	+	+	+	-	-	+	+	-	+	-	-
84	+	+	+	+	-	-	+	-	-	-	-	-
77	+	+	+	+	+	-	+	+	-	-	-	-
396	+	+	+	+	-	-	+	+	-	-	-	-
82	+	+	-	+	-	-	-	-	-	-	-	-
31	+	+	+	+	+	+	-	-	-	-	-	-
170	-	-	-	+	+	+	+	-	-	-	-	-
214	+	-	-	+	+	+	-	-	-	-	-	-
379	+	+	-	+	+	+	-	-	-	-	-	-
315	-	-	-	+	+	+	+	-	-	+	-	-
168	-	-	-	+	+	-	+	-	-	-	-	-
335	-	-	-	-	-	-	+	+	+	+	+	-
181	-	-	-	-	-	-	+	+	-	+	-	-
179	-	-	-	+	+	-	+	+	-	-	-	-
196	-	-	-	+	+	+	+	+	+	+	-	-
Ex. I	+	+	-	-	-	-	-	-	-	+	+	+
Ex. II	+	+	-	-	-	-	-	-	-	+	+	+
75	+	-	-	+	-	-	-	-	-	+	+	-
398	-	-	-	+	+	+	+	-	-	+	+	+

Of these cocci, therefore,

(1) Nine react in such a manner that they may be regarded as probably type I cocci.

(2) Five as probably type II.

(3) Two as probably type III.

(4) Three as probably type IV.

(5) Four are equivocal in their reactions.

These cocci have also been submitted to the absorption test and the majority of them qualify definitely as belonging to types I, II, III, or IV.

Summarising the agglutination results, then, 346 of the 356 cocci react with one or more of the type sera of Gordon. Ten specimens did not agglutinate. Included in the ten which fail to react are two which were received as suspensions and two which were ruled out owing to their reacting in presence of 1/100 dilution and no higher dilution of any serum.

Revising the results, one may say that only eight out of 354 failed to give an agglutination—an experimental error of 2·2 per cent.

In order further to control the findings obtained by the agglutination test as applied to the investigation of spinal strains of the organism, it has now been the practice of the Central Laboratory for some time past, to verify these by means of the absorption of agglutinin reaction. The details of the technique of this reaction I shall here not discuss as this is fully described in another paper¹.

In this series of observations, 107 cocci were completely investigated, being tested each with all of the type sera. Of these 107 cocci, 101 qualified definitely as belonging to one or other of the types defined by Gordon; six could not be so placed, but of these three had died before the investigation was completed.

This enquiry brought to light an interesting point in respect of type II. Absorption showed that the sera produced by certain cocci agglutinated all the representatives of the group, but was not absorbed by contact with all of these. Other strains gave sera which were less potent in that they did not agglutinate so many strains to the full dilution of 1/400. They did, however, agglutinate both the organism used for immunising the animal and a number of other strains provisionally classed as type II to the full titre, and they were absorbed by the majority of the members of the group.

Type II appears to include a complex sub-group and shows considerable variation among the cocci comprised therein. A type II serum can be produced which is suitable for the agglutination test in that all the members of the group are agglutinated by it, but such a serum is absorbed only by a limited number of specimens of type II cocci.

The investigation, by the absorption test, of cocci provisionally classed as type II is therefore a difficult procedure and more than one serum must usually be absorbed before the organism can be definitely placed.

Ten strains provisionally classed as type II cocci and available for examination at one time, were used for absorbing three different type II sera. The results obtained in the experiment are summarised in Table IV which follows.

One of the cocci absorbed all three sera, it would be suitable for the preparation of an agglutinating serum; seven absorbed two of the sera, and two absorbed only one.

¹ The differentiation by means of the absorption of agglutinins test of the types of meningococci obtained from the cerebro-spinal fluid of cases during the current outbreak of cerebro-spinal fever. *Journ. Roy. Army Med. Corps*, July, 1917.

TABLE XIV.

	Coccus	H. serum	Mi. serum	D. serum
(xxvi)	Ou.	-	+	+
(xxxi)	Wy.	-	+	+
(v)	D.	-	-	+
(xvi)	F.	+	-	-
(iv)	Ja.	-	+	+
(xxix)	New.	+	+	+
(i)	A.	+	+	P.
	F ¹ Har.	+	+	P.
(xxviii)	Eng.	-	+	+
(xxxii)	Clen.	-	-	+

+ = Complete or marked saturation.

- = No saturation.

P. = Result equivocal.

By employing them for immunising animals, a coccus which produces a serum similar to that designated H in the above table, one obtains a serum suitable for the agglutination of type II cocci but unsuitable for the absorption test.

In testing cultures, especially those of naso-pharyngeal origin, the least specific (i.e. most difficult to absorb) serum should be used in order that we may be assured that cultures are not being discarded because of an ultra-specificity of the serum employed.

For similar reasons when we are doing absorption with cocci provisionally classed as type II the reaction should always be performed in duplicate, (a) with the most "specific" and (b) with the least "specific" type II sera available.

CONCLUSION.

The results reviewed in this section indicate that the organisms responsible for the current outbreak of cerebro-spinal fever amongst the military forces, are comprised, with remarkably few exceptions, in the four types of meningococcus defined by Gordon.

It might conceivably be contended, however, that the agglutination test does not serve to distinguish the meningococcus from other gram-negative cocci that are not infrequently found in the naso-pharynx, and which are so like that organism both in morphological characters and cultural reactions as to be indistinguishable from it thereby.

While engaged in studying the spinal strains of the organism by means of the absorption of agglutinin test, I took the opportunity of examining a number of specimens of gram-negative cocci, obtained from

the naso-pharynx, which had been regarded as meningococci because of their agglutination reactions. In respect of agglutination and absorption of agglutinin these cocci behaved as did the strains of spinal origin.

When examining the naso-pharynx of a large number of men not infrequently there are obtained cultures of organisms closely resembling the meningococcus, which fail to react under standard conditions with any of the type sera. Are these meningococci or are they not?

The investigation of a consecutive series of 40 such cocci has been completed and it was found that none of them absorbed the agglutinin from any of the four type sera. In examining these, the standard technique was used and each coccus was tested against five sera—type I, type III, type IV and two specimens of type II—to eliminate possible error due to the complexity of type II.

Some of the pharyngeal cocci which fail to absorb the type sera may, when inoculated into animals, produce agglutinins which in high concentrations agglutinate certain strains of meningococci. This is especially liable to occur, if the process of immunisation be prolonged. It might, therefore, be suggested that such organisms could in course of time evolve into meningococci.

Up to the present, however, they have not been, in our experience, encountered invading the meninges with sufficient frequency during the current epidemic to justify their being regarded as pathogenic. It seems, I think, inadvisable—in the present state of our knowledge at least—to accept a hypothesis, based on group agglutination, that the evolution of pathogenic from non-pathogenic organisms, if it occur at all occurs sufficiently rapidly to invalidate the employment of serological methods in the identification of micro-organisms of known pathogenic significance.

Surely the most ardent evolutionary hypothesisist would not insist that the old world apes must needs be endowed with the intellect and other attributes of man because the serum of those animals gives a positive reaction with anti-human precipitin?

THE CEREBRO-SPINAL FEVER EPIDEMIC OF 1917 AT X DEPOT.

By J. A. GLOVER, M.D., D.P.H., *Captain R.A.M.C.*

(With 3 Charts.)

..... its (*i.e.* the then undiscovered meningococcus) selective property for the meninges must certainly be greatly influenced by the insanitary atmosphere that always hovers over large collections of men, by the fatigue, and by the depression of rigid discipline, and by the impaired vitality ensuing from the first entry into a monotonous existence.

Col. DIMMOCK, I.M.S.

Written in 1884 on an epidemic
of cerebro-spinal fever in India.

I. GENERAL DESCRIPTION.

SET 600 feet above sea level, on the highest part of a chalk plateau, and surrounded by open common lands, the depot is a pleasant spot in summer. Unprotected in any way from the east, or indeed from any other wind, there are few bleaker spots in the south of England in the frosts and blizzards of such a winter as that of 1917. Small wonder then if recruit and returned Expeditionary man alike felt justified when, in draughty huts, they closed all windows as tightly as the contractors would permit, and enjoyed the very closeness of their overcrowded atmosphere.

It is an interesting fact that if the beds of a heavily infected hut be charted, the carriers lie usually thickest round the stove, no doubt owing to the nightly gathering round the only warm, and certainly the stuffiest portion of the hut.

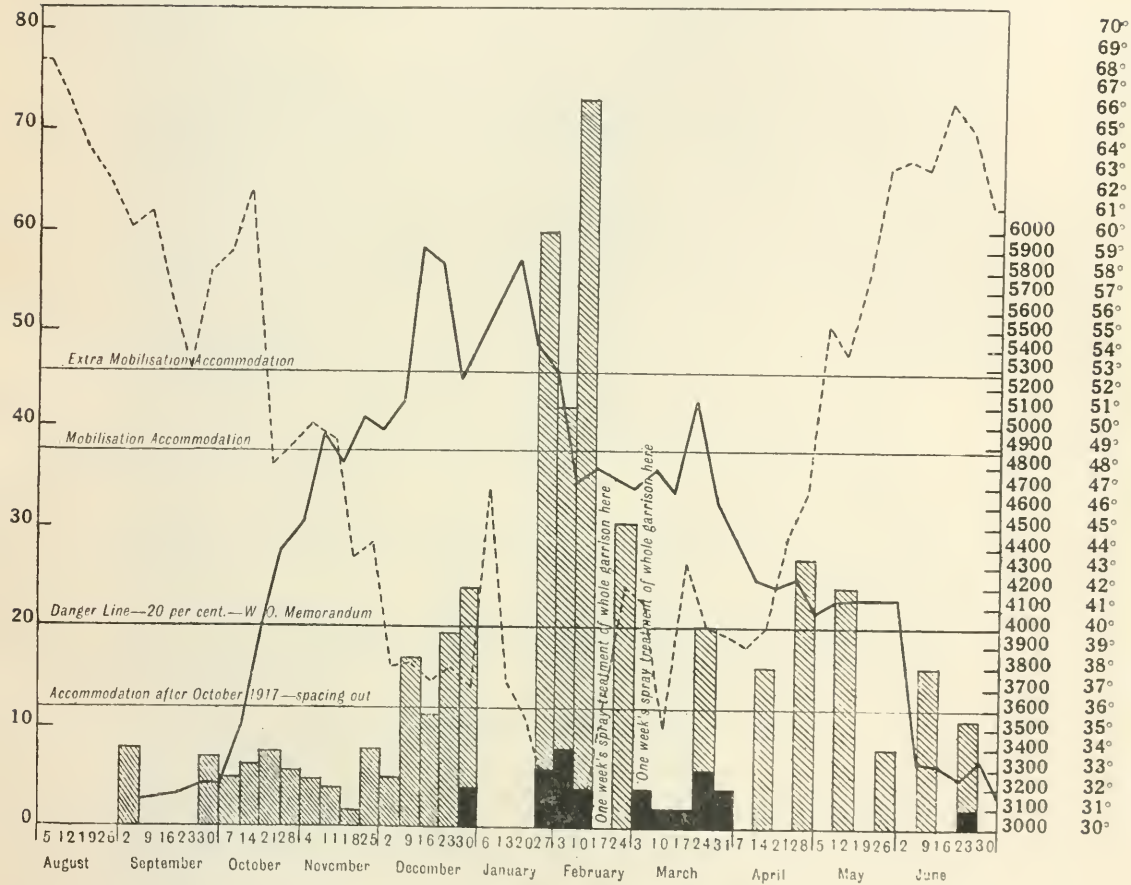
In peace-time the depot is housed entirely in its permanent barrack-rooms and has a normal population of some 800 men. Since war began, its accommodation has been increased to fivefold by huts, and the population has sometimes exceeded sevenfold its peace population.

The depot receives straight from civil life the recruits of five infantry regiments, hereinafter called A, B, C, D, E, in order of both seniority and strength. These recruits it trains for three months intensively,

CHART A. *Cerebro-Spinal Fever. 1917 Epidemic in "X" Depot.*

Percentage Carrier Rate—shaded columns—shown in relation to:

1. Incidence of cases—black columns ■ = 1 case.
2. Ration Strength of Depot shown thus ———
3. Mean Weekly Temperature—broken line thus - - - - -



and they are then drafted to the respective reserve battalions of the five regiments, in various places. Most of the population is therefore floating, it abides three months, and passes. There is, however, a permanent staff of instructors, and two trained soldiers are in each barrack-room or hut. This arrangement may have an important bearing on the transmission of the infection from season to season.

II. PREVIOUS OUTBREAKS OF THE DISEASE.

Cerebro-spinal fever first appeared at the depot in the early part of 1915, following a period of severe overcrowding due to the recruiting situation of that time.

Nineteen cases occurred at the depot, with eleven deaths, followed by ten cases with six deaths in the various barracks of the reserve battalions and in a camp fed by the depot.

No differentiation of types of the meningococci was attempted in these cases, as Lt.-Col. Gordon's work was not then published.

In February, 1916¹, cerebro-spinal fever reappeared at the depot, and between February and April seven cases occurred. These were all due to the Type II meningococcus. The patients all recovered.

From the occurrence of the first case, a very thorough investigation not only of contacts of the cases, but also of contacts of the positive contacts, was carried out by Capt. Flack. This revealed a *contact* carrier-rate of

21 % in February (113 men examined contacts of one case),

9 % in March (219 men contacts of four cases),

20.5 % in April (39 contacts of one case), and

6 % in May (60 contacts of one case).

In every case and in every carrier meningococcus Type II was found.

The undisputed predominance of Type II in cases and contacts is most striking.

All positive contacts thus discovered were forthwith isolated. No cases occurred during this season in the reserve battalions.

III. BEFORE THE EPIDEMIC OF 1917.

THE AUTUMN OF 1916.

In August a large non-contact sample was taken by Capt. Flack by swabbing 121 men who had been three months in the depot. He found a carrier-rate of 10 %, an unexpectedly high figure, especially for the time of year.

He therefore decided to keep a continuous watch upon the carrier-rate by means of large weekly swabbings, as he anticipated a return of the disease in the following winter.

The aggregate swabbings in September showed a carrier rate of 5 %, in October 6 % and in November 4.6 %. So far all seemed well.

¹ Capt. M. Flack, *Special Report, Medical Research Committee*, No. 3.

The depot was, however, rapidly filling up, as a result of the operation of the National Service Acts, and in the first week of November was for the first time above its "mobilisation" accommodation.

On December 4th a number of Aylwin huts were occupied for the first time. On December 9th the overcrowding was at its zenith, over a thousand men more than the ordinary "mobilisation" strength and 600 more than the "extra mobilisation" strength being accommodated in drill sheds, etc., as well as the Aylwin huts.

On December 11th, an epidemic of German measles was fairly established, and from this date carriers detected by sampling were isolated in huts in the depot, instead of being sent to the carrier centre, which was full.

The carrier-rate, which had kept beautifully low during November (5 % on November 29th), now commenced to rise in ominous fashion; on December 6th it was nearly 17 %, on the 21st 19 %, just under the 20 % danger line as laid down in the War Office memorandum.

The carrier-rates are shown upon the Chart A and details of the non-contact samples before the epidemic are seen below.

Period before the commencement of the Epidemic.

Date	Non-contact sample or contact	No. swabbed	No. positive	Carrier rate %	Types			
					I	II	III	IV
Sept. 7	Sample	127	10	8	3	3	—	4
" 14	"	123	1	·75	—	1	—	—
" 25	"	119	7	6	—	5	2	—
Oct. 2	"	120	5	4	1	3	1	—
" 9	"	139	9	6·5	4	4	1	—
" 16	"	118	9	7·5	4	4	1	—
" 23	"	117	7	6	2	4	1	—
" 30	"	121	6	5	—	3	3	—
Nov. 5	"	90	3	3·25	2	—	1	—
" 13	"	115	2	1·75	2	—	—	—
" 22	"	113	9	8	3	1	5	—
" 29	"	120	6	5	2	3	1	—
Dec. 6	"	120	20	16·75	2	17	—	1
" 13	"	119	14	12	12	2	—	—
" 21	"	125	24	19·25	3	20	1	—
" 29	"	91	10	11	1	9	—	—

IV. THE EPIDEMIC.

Just at the end of the year, all the requisite factors for an epidemic of cerebro-spinal fever were present, cold weather, severe overcrowding, a high carrier rate, and a population rendered susceptible by youth,

by strange surroundings and methods of life, and by the depressing effects of nostalgia combined with the malaise of anti-typhoid inoculations and vaccination performed before acclimatisation to their new circumstances was established.

The first case was diagnosed on December 29th, and was almost immediately followed by one other. The contacts (73) of these two cases showed carrier rates of 23 and 27 % respectively. Both were due to Type II meningococcus, seventeen out of twenty positive contacts carrying the same type.

No further cases occurred for three weeks, and owing to the unfortunate illness of Captain Flack, no samples of non-contacts were taken during this period. Captain W. Allan then took over the work for a few weeks, until he also became ill; his observations are those recorded from January 24th to February 19th inclusive.

During the same three weeks the Aylwin huts, which had been leaking badly, were given up, and five hundred men were sent away from the depot to ease the overcrowding. Influenza of a mild type and bronchial catarrhs became increasingly prevalent.

Between January 24th and February 2nd seven cases occurred in succession, three of them being fulminating cases, the patients dying within twenty-four hours of the onset. Of these cases, 185 contacts were swabbed showing a carrier-rate of 46 %.

The first of these cases was due to Type I meningococcus, the rest were all due to Type II.

The contacts of the first case showed a carrier-rate of 43 %, those of the second reached the appalling rate of 71 %, the hut containing 32 men, one of whom was the patient (Type II), ten of whom were carriers of Type I, eleven of Type II, one of Type III. Six of the nine remaining had organisms resembling meningococci which failed to agglutinate with the standard sera, only three were above suspicion.

A sample of non-contacts taken on February 5th showed the even higher rate of 72.5 %, the trained soldiers being almost as heavily infected as the recruits. The period of this group of cases exactly coincides with the great frost—the severest weather experienced in England since 1895.

It was now obvious that a severe epidemic was threatened, and the question of swabbing the whole garrison and isolating all carriers was discussed and negatived on account of the magnitude of the work, and the practical certainty that half the population was carrying.

Instead, the following measures were taken: parades were shortened,

inoculation and vaccination stopped, men were spread out as much as possible (numbers were now slightly below "mobilisation" scale of accommodation), a certain number of windows in each hut and room were fixed open, and the crowding of Y.M.C.A.'s etc., was limited.

It was also determined to subject the entire population to treatment with Levick steam sprays, using 1 % zinc sulphate dissolved in normal saline. Four huts were adapted using eight Levick sprays, each hut having a small central portion partitioned off to form an inhaling chamber of some 1800 cubic feet and containing two Levick sprays, the portions of the hut on either side served respectively as an antechamber to prevent loss of vapour, and as a cooling-off room. The latter was warmed by stoves and the men remained in it seven minutes after being seven minutes in the inhaling chamber. Every man in the depot was thus treated for seven consecutive days.

After a two days' interval the camp was systematically sampled.

Results of Sample Swabbings after One Week's Spraying.

Date	Regiment	No. swabbed	No. positive	Carrier rate %	Types			
					I	II	III	IV
19. 2. 17	A	50	15	30	0	14	0	1
19. 2. 17	B	50	11	22	1	10	0	0
22. 2. 17	C	50	12	24	2	9	0	1
22. 2. 17	D	50	18	36	8	8	0	2
24. 2. 17	E	50	21	42	4	17	0	0
Total		250	77	30.8	15	58	0	4
				Total number swabbed	250			
				Total number positive	77			

Feb. 19-24. Carrier rate (after spraying) 30.8 %.

The general carrier-rate, therefore, had fallen from over 60 % to 30.8 %. This was encouraging but still too high, and all the garrison were again submitted to a week's course of spraying beginning February 28th, 1917.

The general use of the steam spray was then discontinued and on March 7th only freshly joined recruits were sprayed during their first week in the depot.

On March 22nd another sampling was done, the result being:

Date	Regiment	No. swabbed	No. positive	Carrier rate %	Types			
					I	II	III	IV
22. 3. 17	B and C 23 of each	46	9	20	5	4	0	0

The contacts of these cases swabbed upon the same day showed a carrier rate of 31 %.

The last two cases of the epidemic occurred on the same day, March 27th, and in the same hut. This was the only occasion in which any apparent connexion between patients occurred, but the population of this hut showed a carrier-rate of only 7 %, but two carriers of Type II being discovered, although all were twice examined on different days. This was in regiment "C."

Nine cases occurred during March, making nineteen in all during the epidemic; there was also one case in which the diagnosis was doubtful.

Of the undoubted cases, one only was due to meningococcus Type I, thirteen were due to Type II, and in five cases growth was not obtained, owing either to the death of the patient in the fulminating cases, or to the chilling of the specimen taken late at night in the cold, and on the long journey to the laboratory.

Cases were fairly evenly distributed amongst the regiments in proportion to their strength. "C," a Scottish regiment, had comparatively low carrier-rates throughout, whilst "D," an Irish regiment, had high carrier rates and no cases at all.

One additional sporadic case (due to Type I) occurred on June 20th.

V. CONTACTS.

During the time of the general spraying, examination of the contacts of six cases was not done, as all were being treated, and in view of the high carrier-rate prevailing throughout the depot, which was quarantined, isolation of these contacts was not practised.

Of the other fourteen cases (including the doubtful case) 426 contacts were examined, 145 of whom were found positive: 71 % of the positive contacts carried Type II meningococcus.

It is, however, both interesting and important to note, that if the total contact swabbings during the epidemic be compared with the total non-contact sample swabbings during the period of the epidemic, it will be found that the average carrier-rate is the same, 34 % in each case. It will also be seen that these totals show nearly the same proportion of Type I and Type II.

The following table shows a summary of the three periods:

I. The autumn months prior to the occurrence of the first case on December 29th.

II. The epidemic period December 29th to the end of March, both contact, and non-contact.

III. The quarter April 1st to June 30th immediately following the epidemic during which samples have been taken at regular intervals.

Summary of Periods.

	Period	No. swabbed	No. found positive	Carrier rate %	Types			
					I	II	III	IV
I.	September to Dec. 29th. All non-contact	1877	142	7.5	41	79	17	5
II.	Period of epidemic, December 29th to March 31st. Contacts (Samples) non-contacts	426 336	145 115	34 34	38 31	103 78	2 2	2 4
III.	Quarter after epidemic, April 1st to June 30th	669	102	15.3	16	76	9	1
Totals, Sept. to June 30th		3308	504	15.2	126	336	30	12

Details of all examinations during Epidemic Period.

Date	Description	Number swabbed	Number positive	Carrier rate %	Types				Percentage carrying gram-negative cocci failing to agglutinate
					I	II	III	IV	
Dec. 29	Contacts with a case of Type II	30	7	23	—	5	1	1	47
Jan. 4	Contacts with a case of Type II	44	13	27	1	12	—	—	41
" 24	Contacts with a case of Type I	30	13	43	2	11	—	—	40.5
" 26	Contacts with a case of Type II	31	22	71	10	11	1	—	19.5
" 29	Contacts Type II case	28	14	50	4	10	—	—	25
" 31	Contacts case Type unknown	26	6	23	—	6	—	—	73
Feb. 2	Contacts Type II case	32	10	31	5	5	—	—	46.75
" 6	"	26	17	65	2	14	—	1	8
" 5	Non-contacts sample	40	29	72.5	11	16	2	—	20
" 19	"	250	77	30.75	15	58	—	4	5.25
March 17	Contacts II case	26	2	7.75	—	2	—	—	15.25
" 22	"	29	7	24	6	1	—	—	21
" 22	"	68	20	29.5	7	13	—	—	25
" 23	Contacts with a case Type unknown	28	12	42.75	1	11	—	—	14.25
" 22	Non-contacts sample	46	9	20	5	4	—	—	32
" 28	Contacts II cases (2)	28	2	7	—	2	—	0	7
Totals	Contacts ...	426	145	34	38	103	2	2	
	Non-contacts ...	336	115	34	31	78	2	4	

VI. CARRIERS OF GRAM-NEGATIVE DIPLOCOCCI, WHICH ARE CULTURALLY INDISTINGUISHABLE FROM MENINGOCOCCI BUT WHICH FAIL TO AGGLUTINATE WITH ANY ONE OF THE FOUR STANDARD SERA.

A careful record has been kept of the percentage of men who carry organisms resembling on culture the meningococcus.

A comparison of the carrier rates of such men with the carrier rates of carriers of the serologically proved meningococcus shows some interesting points.

During the autumn period prior to the epidemic the proportion of agglutinating organisms to non-agglutinating strains was quite small, usually about one-sixth, often less and going as low as one-twentieth.

During the epidemic period, as the carrier-rate rose, this proportion rose rapidly too, in non-contact samples equally with contacts, the agglutinable cocci now forming two-thirds, three-quarters, or even more of the whole.

This proportion was maintained for some time and sank much more slowly than it had risen. The proportion was not diminished by steam spray treatment. Thus, in the sample immediately before the treatment was commenced, of forty men thirty-seven (92.5 %) carried organisms resembling the meningococcus; of these thirty-seven, twenty-nine (72.5 % of the whole) agglutinated (eleven Type I, sixteen Type II, two Type III), whilst eight failed to agglutinate.

In the sample taken two days after the first week of spray treatment of 250 men, 91 (36.4 %) carried meningococcus-like organisms. In seventy-seven (30.75 %) agglutination was positive (fifteen Type I, fifty-eight Type II, four Type IV), whilst only fourteen failed.

The steam spray treatment reduced the just and the unjust alike.

These observations seem to lead to one important practical point, cultural characteristics are a much more reliable guide with a high carrier-rate, and during an epidemic, than they are with a low carrier-rate in a non-epidemic period.

The method of diffusion of these non-agglutinating organisms is probably exactly similar to that of the meningococcus.

Flavus agglutination has been given throughout the Chart and Table as negative.

CHART B. 1917 Epidemic Cerebro-Spinal Fever. "X" Depot.

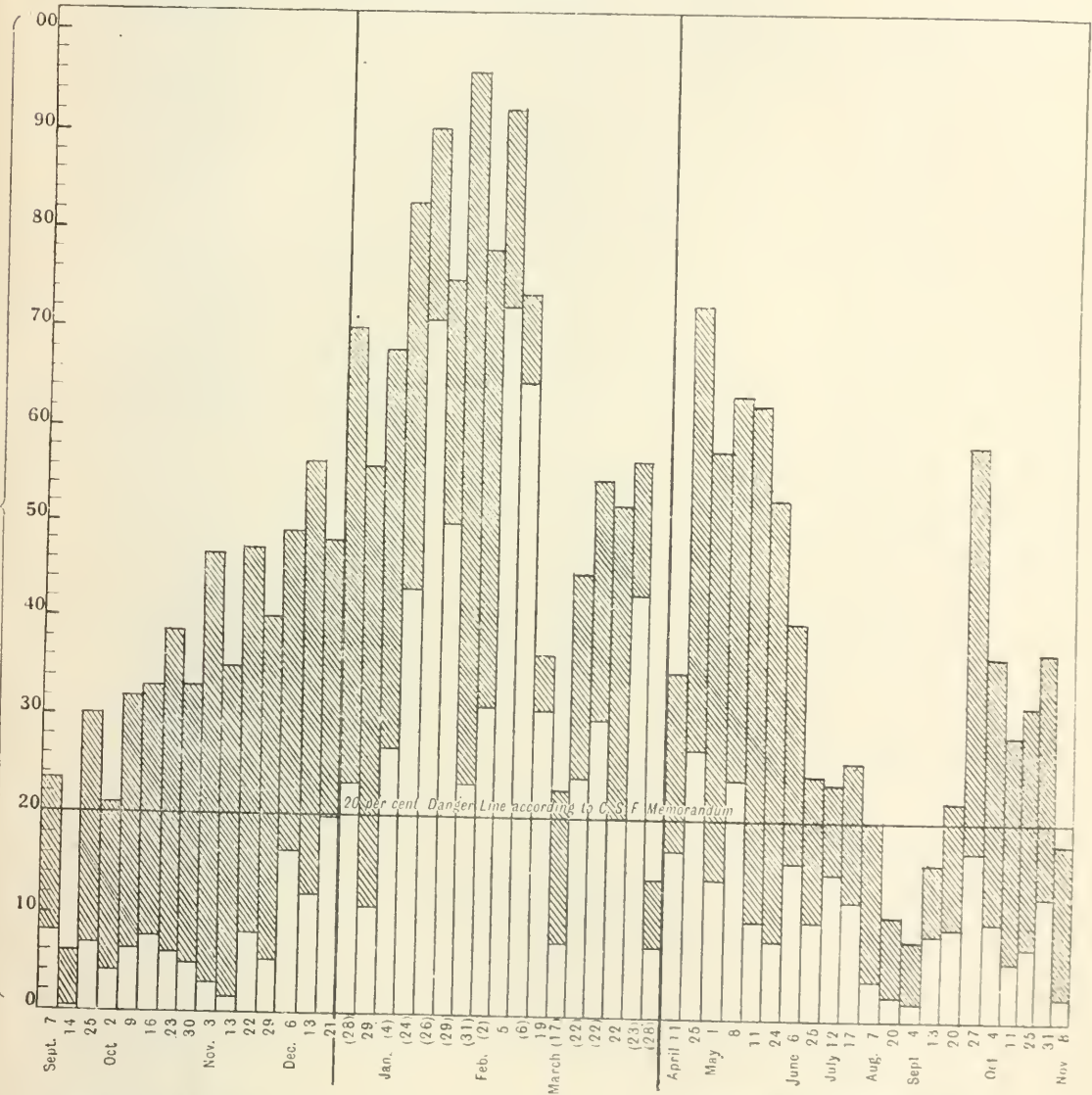
Relative percentages of:

1. Carriers of gram-negative cocci morphologically indistinguishable from meningococci but not qualifying serologically as such—shaded columns.
2. Carriers of meningococci serologically proved—plain columns.

Showing the greatly increased proportion of the latter during the Epidemic Period.

Period of low carrier rate
Autumn

Epidemic period



Dates in parentheses = contacts of cases, thus: Jan. (4).

Plain dates = sample swabbings.

Carrier-rates per cent. of:

- I. All organisms morphologically resembling the meningococcus.
- II. True meningococcus, *i.e.* those which agglutinate with any one of the four standard types.
- III. Inagglutinables with standard sera.

		I.	II.	III.	
Date of swabbing		Contacts (usually 30) or non-contacts usually 100-120 men	Percentage carriers of all organisms. resembling meningococcus	Real carrier rate: percentage carrying agglutinable meningococci	Percentage carrying non-agglutinable cocci
Sept.	7	Non-contacts	23.5	8	15.5
"	14	"	6	7.5	5.25
"	25	"	30	7	23
Oct.	2	"	21.5	4	17.5
"	9	"	32	6.5	25.5
"	16	"	33	7.5	25.5
"	23	"	38.5	6	32.5
"	30	"	33	5	28
Nov.	5	"	46.5	3.25	43.25
"	13	"	35	1.75	33.25
"	22	"	47	8	39
"	29	"	40	5	35
Dec.	6	"	49	16.75	32.25
"	13	"	56.5	12	43.5
"	21	"	48	19.25	28.75
"	29	"	56	11	45
Epidemic period begins here					
Dec.	29	Contacts	70	23	47
Jan.	4	"	68	27	41
"	24	"	83.5	43	40.5
"	26	"	90.5	71	19.5
"	29	"	75	50	25
"	31	"	96	23	73
Feb.	2	"	78	31.25	46.75
"	6	"	73	65	8
"	5	Non-contacts	92.5	72.5	20
"	19	"	36.5	30.75	5.25
March	17	Contacts	23	7.75	15.25
"	22	"	45	24	21
"	22	"	54.5	29.5	25
"	23	"	57	42.75	14.25
"	22	Non-contacts	52	20	32
"	28	Contacts	14	7	7
Epidemic period ends					
April	11	Non-contacts	35	17	18
"	25	"	72.5	27	45.5
May	1	"	58	14	44
"	8	"	64	24	40
"	11	"	63.5	10	53.5
"	24	"	53	8	45
June	6	"	40	16	24
"	25	"	25	10	15

VII. AFTERMATH OF THE EPIDEMIC.

The carrier-rate of the depot remained high up to August when it dropped suddenly and reached the nadir of 2 %, which may be regarded as the irreducible minimum.

In July it had reached 15 % and large drafts being sent to the various reserve battalions, some of whose barracks were in a very crowded condition, a rather alarming series of events occurred.

At the barracks of "C" regiment (which, it will be remembered, had been characterised by a somewhat low carrier-rate and its correct proportion of cases (4) to its strength at the depot) a sub-epidemic of three cases with two deaths occurred in September.

All the cases were in the same block of the barracks, an old building of bad design and with bad ventilation. All the patients had come within a few weeks from the depot at X. All suffered from the invasion of Type II meningococcus. The contacts and a large sample showed a carrier-rate of 11 %.

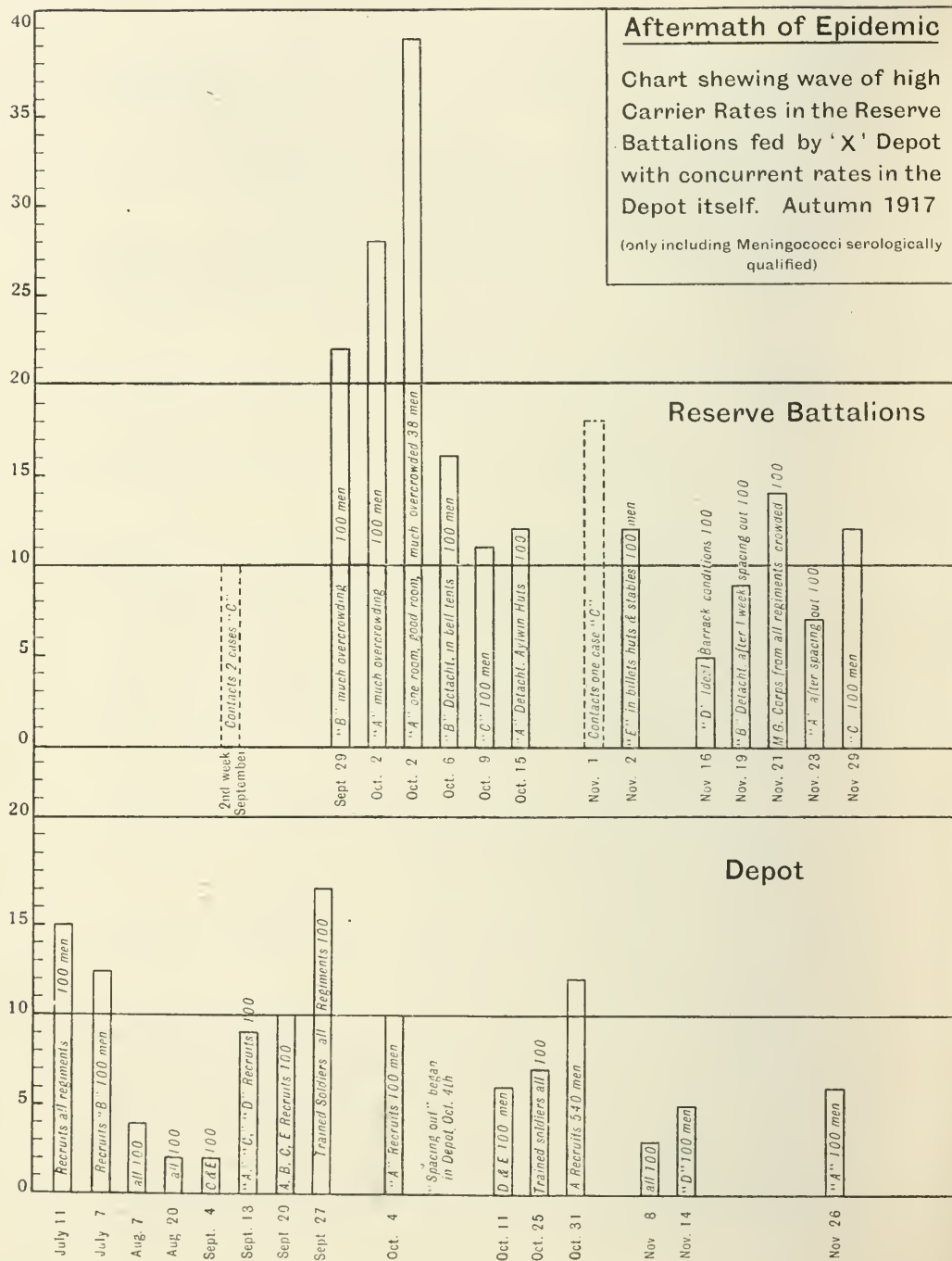
After these cases, samples were taken of the reserve battalions, of "A" and "B" regiments, whose barracks were 22 miles apart. In both there was considerable overcrowding. The barrack-room sample of "A" showed the alarming figure especially for early October of 28 %, one large room reaching the appalling rate (for the season) of 38.75 %. "B" showed the high figure for barrack-rooms of 22 % (September 29th) and of 16 % for men under canvas (October 6th). On each occasion, 100 men were sampled.

The reserve battalion of "D" under ideal barrack-room conditions (November 16th) gave a carrier-rate of 6 %, and the reserve battalion of "E" under only fair conditions, somewhat crowded in a mixture of billets, huts and stables had 13 %.

How closely the meningococcus carrier-rate is correlated to over-crowding is shown not only by the A Chart for the depot (black line), but by the experience with these reserve battalions.

"A" having had to furnish large drafts is now comparatively well accommodated and is almost down to peace strength accommodation (60 feet floor space per man). Beds have been spaced out and the carrier-rate on November 23rd was just a quarter (7 %) of what it was on October 2nd, whilst the large room that had the 38 % carrier-rate, when badly crowded, has now but 4.5 %.

Fortunately, no further cases of the disease have occurred attributable to the depot epidemic.



These high carrier-rates have been largely due to an autumnal renaissance of Type I, a phenomenon seen on a smaller scale last year. The three cases, however, were all due to Type II.

VIII. SUMMARY.

(1) There were nineteen cases and eight deaths at the depot during the epidemic.

One doubtful case during this period recovered, and a sporadic case in June due to Type I also recovered; these are not included in the above, nor is one man who went on leave to Blackpool, and developed the disease (Type II) on arrival.

(2) So far the reserve battalions have had three cases only, with two deaths, although a high carrier-rate wave has been detected in two.

(3) All the cases (except the June case) at the depot occurred during exceptionally cold weather. In the chart the cases appear to lie in the trough of a great depression of the curve of mean weekly temperature (broken line). They also follow immediately upon exceptional crowding (black line).

(4) There was a well-marked premonitory rise in the carrier-rate in December 1916, before the first case occurred, and an enormous rise before the epidemic was really established.

The carrier-rate, which was 19.25 % on December 23rd, reached what is usually considered the danger point of 20 % (see War Office Memorandum on Cerebro-spinal Fever, page 2) just six days before the first case occurred.

It would appear that *estimations of the carrier-rate by means of large sample swabbings afford a reliable warning of the imminence or danger of an epidemic.*

(5) The cases in the epidemic were nearly all due to the meningococcus of Type II, the organism present in the outbreak of 1916.

The rise in the carrier-rate was also chiefly due to the increase of carriers of this type. Freshly joined recruits showed few carriers at all and very few Type II carriers when swabbed before having slept in the barracks.

(6) During the epidemic, the carrier-rate among non-contacts was substantially the same as amongst the actual contacts of cases, averaging 34 % for the period, in each case.

(7) The proportion of agglutinable strains to inagglutinable strains of organisms morphologically indistinguishable from the meningococcus increased very markedly during the epidemic period.

(8) The treatment of the whole population by the steam zinc sulphate solution spray (which was carried out daily for two seven-day periods with an interval of a fortnight), was followed by a satisfactory drop in the carrier-rate, and by a temporary cessation of cases on each occasion.

(9) 60 % of the actual patients suffering from the disease were in their first month of service.

40 % of the patients had either been inoculated or vaccinated within seven days of onset (three on the same day); 60 % within a fortnight.

(10) Previous and concurrent epidemics of German measles, influenza and bronchitis had helped (by causing coughing and sneezing and by lowering vitality) to produce the great rise in the carrier-rate, which culminated in the case epidemic.

IX. PROPHYLAXIS FOR 1918.

The carrier-rate in August dropped to 2 %. This was evidently the acceptable time to try to prevent by early measures a return for the fourth time of the disease in the coming winter.

Accordingly, following the lessons, bacteriological and otherwise, of the 1917 epidemic, a scheme was produced by the kind help and co-operation of the Commandant of the depot, and of the Senior Medical Officer. It received the permission and the backing of the authorities, particularly the very kind and prompt help of the D. D. M. S. and the S. S. O. of the District, after it had been submitted to the valuable advice and criticisms of Lt.-Col. Gordon of the Central Cerebro-spinal Fever Laboratory.

It has been carried out in excellent time and awaits, with some trepidation, it must be confessed, the verdict of the coming winter.

The chief prophylactic measures are:

1. Spacing out of beds to a minimum interval between beds of $2\frac{1}{2}$ feet. To do this means a reduction of 1266 beds under mobilisation standard.

The depot, being much less full than last year, the extra accommodation was obtainable, the chief difficulty being that extra huts occupied meant extra fuel, and fuel is issued on a scale of men and not of huts occupied.

This difficulty, however, was soon overcome by the recommendation of the D. D. M. S. and no hut has now more than twenty-four beds compared with thirty-two last year.

The difference in the general amenities of life is very striking, and the comfort and appearance of the huts is much increased, apart from the diminished risk of the spread of any catarrhal diseases.

No barrack-room has now more than twenty-six beds in it.

2. Special ventilation is being arranged, four windows in each hut are being fixed open (15°) with hopper sides, and with a prolongation of the outside weather boarding to give an upward direction to the air and to prevent driving rain and snow from entering.

Four windows are also being fixed slightly open in each barrack-room.

3. During the danger months December to March, anti-typhoid inoculation is to be done in the second month with fourteen days between doses, vaccination is done in the first month, great care being taken, as was done previously, during the period of vaccinia malaise.

4. A hut has been adapted as a special spray chamber with an external boiler and twenty Hine's jets under the direction and supervision of Major Hine himself.

This is capable of spraying 120 men simultaneously and can be filled with spray vapour in two minutes. The atmosphere is not vitiated by the fumes of the burning spirit as with the Levick spray; it remains quite cool and pleasant.

It is proposed to spray each of the five regiments in rotation every day for six days once a month, if any rise takes place in the carrier-rate sufficient to appear a warning.

It is also proposed to spray all recruits on entry for their first six days, and, if the carrier-rate of the depot be higher than that of the reserve battalion (at present it is lower), to spray all drafts leaving the depot.

5. The medical inspection premises are to be enlarged. Every recruit has to go to these at least four times for examination, vaccination and two inoculations. During the last epidemic, the premises, being most inadequate, were always very crowded, and probably a fruitful source of infection. Practically all the nurses and orderlies working in them, became temporary carriers during the epidemic.

6. A large sample swabbing (100 men) is taken weekly and affords a guide to the current carrier-rate.

EDITORIAL NOTE

Owing to lack of space, the two following papers have to be held over for publication in the next number of the *Journal of Hygiene*:

GLOVER, J. A. Observations on the Meningococcus Carrier-Rate in Relation to Density of Population in Sleeping Quarters.

WALKER, E. W. AINLEY. A Contribution to the Study of Meningococci.

OBSERVATIONS ON THE MENINGOCOCCUS CARRIER-RATE IN RELATION TO DENSITY OF POPULATION IN SLEEPING QUARTERS.

BY CAPTAIN J. A. GLOVER, R.A.M.C.

(With 3 Charts.)

THE carrier-rate of epidemic strains of the meningococcus in any military unit is of great importance from the point of view of prevention of cerebro-spinal fever for three reasons:

(1) A high carrier-rate is a storm warning of an approaching outbreak of cases.

(2) It is an index of the danger of cases occurring amongst the civil population, when men from the unit are billeted upon them, or return home on leave, or on demobilization.

(3) It is an admirable index of the hygienic conditions under which the unit lives. In other words, it appears to vary directly with the degree of overcrowding.

Elsewhere I have tried to deal with the first proposition, the second is sufficiently obvious to stand by itself, it is the third proposition that I hope to prove, and I propose in these short observations to deal only with the carrier-rate in relation to overcrowding, and the extraordinary improvement in the carrier-rate, which follows upon the simple remedy of increasing the distance between beds.

Johnson and Treadgold working on Salisbury Plain in the early days of the War, pointed out that the number of positive contacts found in connexion with a case of the disease was directly proportionate to the degree of overcrowding in the conditions of contact, i.e. of housing.

Marked overcrowding was accompanied by a carrier-rate *amongst contacts of cases* of 20 per cent.

Moderate overcrowding by one of 11 per cent., whilst when there was no overcrowding the carrier-rate was six per cent.

Now these figures, which refer entirely to contacts of cases, practically agree with the results of the examinations of more than 4000 *non-contacts*, soldiers of the London District, who for six months have never been near cases of cerebro-spinal fever.

The determination of the carrier-rate of each unit has been done by the examination of samples of 100 men, and, as a rule, the samples examined are not of men taken haphazard from the battalions, but a certain number of sleeping rooms, or huts are taken, and as far as possible *every* man sleeping in these rooms is examined.

A bed chart, showing the relative positions in which the men sleep, is also used, and proves most useful as an index of the amount of cross infection which obtains. Thus, if one finds a group of three or four carriers of the same type of meningococcus in contiguous beds or in the same corner, and other groups of carriers of either meningococci, or organisms resembling meningococci, in other parts of the room, it is pretty certain that cross infection is rife, and such grouping of carriers is more suggestive of unhygienic conditions than the same number of carriers more uniformly distributed.

It was found, for example, that *amongst non-contacts* severe overcrowding in a unit was usually accompanied by a carrier-rate of about 25 per cent. in that unit; moderate overcrowding by one of 12 per cent.; whilst a unit with no overcrowding averaged 5 per cent.

The limits of severe overcrowding are perhaps a little difficult to define on account of the variation in the class of the accommodation, which included barrack-rooms, rooms adapted from empty houses, breweries, loose-boxes and pavilions, huts of different patterns and sizes, and tents, but by far the most important conditions influencing carrier-rate are:

- (1) the distance between the beds,
- and (2) the ventilation.

We may therefore define severe overcrowding as existing, when the beds in the sleeping quarters are less than one foot apart.

It should be observed that the distance between the beds is a more valuable index than floor space. Thus there are two patterns of the ordinary huts 60 feet long, one type 60' \times 20', the other 60' \times 15'.

Now, on mobilization standard, 30 men can be accommodated in the first and 22 men in the second type, mobilization standard allowing 40 square feet per man of floor space.

The beds in the large pattern with 30 men will be 1 foot 4 inches away from each other (i.e. after allowing for the stove on one side, as is usual), whereas the beds in the smaller type with 22 men will be more than 2 feet 6 inches apart, the floor space in both cases being identical. There is no doubt that if mobilization standard be adhered to, the smaller type is the safer. Unfortunately, the 60' \times 15' type with its

long sides offers an apparently irresistible temptation to overcrowding and is almost invariably crowded above its 22 standard and then it is, of course, more dangerous than its larger brother.

In adapted buildings, such as breweries, race-course pavilions and school-rooms, floor space may be very misleading, as obstructions and fixtures may compel the men to lie close together, as may also deficient heating, or the issue of too few blankets compel them to roll up in the same blankets.

It should be remembered that the standard accommodation was laid down by Royal Commission in 1861, after the horrors and scandals of the Crimean War, and was fixed at 60 square feet of floor space, 600 cubic feet air space, and three feet distance between beds.

In the extreme pressure following the outbreak of this War, permission was granted to use a mobilization standard of 40 square feet floor space and 400 cubic feet air space, but nothing was said as to the distance between the beds. It looks like a simple deduction that if the floor space be reduced from 60 square feet to 40, the distance between the beds will be reduced from 3 feet to 2 feet, but it actually happens that in the ordinary barrack-rooms or huts, where sleeping is not feasible, except along the sides, the reduction is more than 50 per cent.

The distance between beds in an ordinary barrack-room on mobilization standard averages 1 foot 4 inches, after allowing for fireplaces, cupboards and doors; and any beds over this reduce it very rapidly.

Thus, in an actual instance, where the peace strength of a barrack-room was 24, 47 men were actually sleeping in it, and the beds instead of being $\frac{3 \times 24}{47} = 1\frac{1}{2}$ feet apart actually were less than 6 inches apart.

The carrier-rate here was 22 per cent.

The peace standard is practically attained in one or two units in my district, and may be considered to have as its index a carrier-rate of 5 per cent. or under.

A strict observance of the mobilization standard has scarcely ever in my experience been accompanied by a rise above 10 per cent.

If, with spacing on a peace standard, we have more than 5 per cent. or more than 10 per cent. with mobilization standard, it is probable that there is something seriously wrong with the ventilation.

If the carrier-rate rises above 20 per cent., gross overcrowding is practically certain, and if the season be winter, an outbreak of cases of the disease is probable and vigorous measures are urgently indicated.

Isolated cases may, of course, occur with any carrier-rate.

Here are some instances demonstrating these relations (Autumn, 1917):

Accommodation	Distance between beds	Floor space in square feet	Ventilation	Carrier-rate per cent.
Old Barrack-rooms grossly overcrowded	6 inches	39	Spoilt by Lighting Regulations	22
Good Barrack-rooms	10 inches	Just under 40	do. do.	28
" " "	10 inches	40	do. do.	38
Vat-room adapted	under 10 inches	Irregular obstructions	Bad	28
Superior pattern huts grossly overcrowded	Practically touching	25	Good	20
Aylwin Huts	Irregular	26 (Note 170·5 cubic space)	Porous and draughty	15
Loose boxes overcrowded	Very close	—	Poor	19
Grand stand overcrowded	Beds along one wall within 9 inches	Large but owing to obstructions much space unavailable	Poor; but large cubic space	14
Huts 60' × 15' some overcrowding (averages 27 men instead of 22)	2 feet	35	Fair	10
Same type hut average 28 and lower ground	Under 2 feet	34	Fair	13
Barrack-rooms (old)	3 feet	60	Good	5
Huts 60' × 20'	2½ feet	53	Special	3

Partitions in huts are undoubtedly bad owing to their multiplying the number of corners, and small sub-divisions limit infection, but at the same time focus it.

One partition in a hut, for example, in addition to increasing the difficulties of through ventilation, doubles the number of corners, and carriers are often found sleeping in the corners of huts, owing, no doubt, to the dead-end character of corner ventilation.

Two instances of small sub-divisions focussing infection may be quoted:

A detachment of 101 men are sent into camp to ease the pressure on an overcrowded barrack in London.

They undoubtedly left London with a high carrier-rate. After a week under canvas all were swabbed.

The general carrier-rate was 16 per cent.

Of the 101 men 60 slept in tents (bell single flap) each holding 8 men or less.

Of these the carrier-rate was 11 per cent.

Forty-one men slept in four tents holding nine, ten, and eleven (two tents thus) each.

Of these the carrier-rate was 22 per cent.

Now consider the distribution in tents: out of thirteen tents four were quite clear of carriers; four had only one carrier; three had two carriers. and two had three carriers.

In only one tent was there a mixture of carriers of different types: this tent had three carriers amongst nine men; two carrying Type II meningococcus and one carrying Type I meningococcus; all the other four tents which had more than one carrier bred true, that is, had them of the same type.

Thus we see in a unit with a general carrier-rate of 16, but divided into small communities, the difference in risk to a susceptible individual may be immense according as he may happen to sleep in a tent free of carriers or in one in which three out of the nine occupants are carriers.

Another example is furnished by a detachment sleeping in Aylwin huts, 8 to a hut, in December. Fifteen per cent. was the carrier-rate, and it is somewhat surprising that the figure was not higher as the floor space with 8 men in an Aylwin hut is only 26 square feet and the cubic space only 170·5. These so-called huts are probably safer than they would appear, owing to the porous nature of their material and the numerous leaks, etc., through which copious draughts of fresh air continually pour. They really approximate much more to tents than huts.

Now, on analysing this 15 per cent., it was found that out of twelve huts, three were free of carriers, four had only one carrier, whilst one hut had three carriers amongst its eight men and one had four.

The chances of a susceptible individual contracting cerebro-spinal fever when he is introduced into such a community as the last mentioned hut, half of whom are carriers housed with 26 square feet of floor space and 170·5 cubic feet of air space must be considerable in the month of December, and on the results being reported, the conditions were immediately remedied.

Two examples may be quoted of the interesting effect of partitions in huts and rooms.

The first is a hut of the usual dimensions, 60' \times 20', but of superior construction, and with larger windows of the French window type. It had a partition dividing it into two. In this hut which, on mobilization standard, should have accommodated thirty men, forty-two men were sleeping, eighteen in one side and twenty-four in the other, instead of fifteen in each compartment.

Four of the eighteen on one side of the partition were carriers, two sleeping side by side.

Of the twenty-four in the other compartment, only twenty-two were examined, and of these nine were carriers with no less than six Type I carriers in adjacent beds on one side of the hut. A clearer case of cross infection could not be imagined.

It will be observed that the carrier-rate of one half is 22 per cent., that of the more severely overcrowded half is 37 per cent.

To show the possible influence of a partition in a room under fair conditions, and to demonstrate what can be found out sometimes by examining closely the results of a sample: 100 men of one battalion were examined, the number being made up of fifty men sleeping in cubicles and well spaced out small rooms; none of these men were carriers. Thirty-four of the remainder came from one barrack-room, which was divided by a partition into inner and outer parts with seventeen men in each. The inner portion was the smaller and in one place the beds were only about 1 foot apart. There was one carrier in the outer part and three in the inner. Therefore, although the whole sample showed the excellent carrier-rate of 4 per cent., yet here was a compartment in the barracks with a carrier-rate of 18 per cent., and obviously a source of potential danger.

THE EFFECT OF INCREASING THE DISTANCE BETWEEN BEDS UPON THE CARRIER-RATE.

That Cerebro-spinal Fever is a disease of which the occurrence is favoured by overcrowding has long been accepted—it is probably impossible to find an infectious disease which is not—and, as the infection is almost certainly conveyed in droplets of mucus in the acts of coughing, sneezing, and loud speaking, it is obvious that by increasing the distance between beds the likelihood of infection passing from man to man will be diminished.

This obvious truth, however, hardly prepares us for the extraordinary improvement in the carrier-rate in a unit, produced by quite a small increase in the distance between beds in a short time, even when that unit has already been heavily infected.

Full mobilization standard means in most barrack-rooms and huts a distance between beds of 1 foot 4 inches, and this, even rigidly carried out, is not sufficient to produce a rapid improvement in an already high carrier-rate.

On the other hand, at the present time, the peace standard of 3 feet between beds, even in a heavily infected unit, seemed almost unattainable, owing to the demands on accommodation.

A purely arbitrary standard midway between, was therefore selected, and the beds being $2\frac{1}{2}$ feet across, $2\frac{1}{2}$ feet was selected as the space between. In a $60' \times 20'$ hut this allows twenty-three men (or twenty-four if the stove be not working), to sleep without the side of any bed being less than $2\frac{1}{2}$ feet from that of its neighbours, and each man has approximately 53 square feet of floor space (with twenty-three men).

In a $60' \times 15'$ hut, mobilization standard, if strictly enforced, only allows twenty-two men, so that with such a hut, there is no difficulty in attaining the desired distance.

A similar provisional standard was attempted with barrack-rooms, and in every case attempts were also made to increase the ventilation, by the fixation of windows, some being converted by wooden sides to fixed hopper ventilators.

The improvement was remarkable and occurred in all classes of accommodation, Aylwin huts, and loose boxes, as well as in the better kinds of housing, although not quite to the same extent.

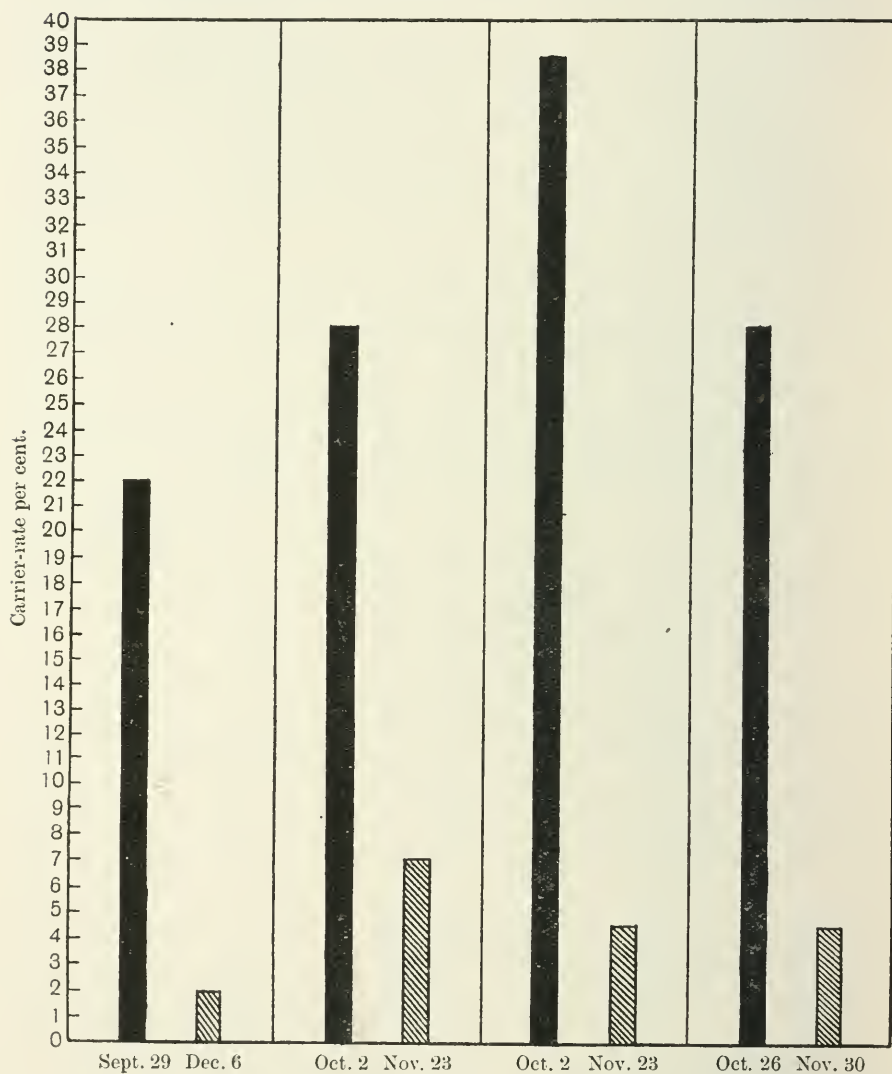
The greatest improvements were shewn in barrack-rooms and in the $60' \times 20'$ huts.

The very remarkable results with barrack-rooms are shewn upon the Chart A.

*Effects of spacing out on the Carrier-rate in severely overcrowded
BARRACK ROOMS.*

Carrier-rate per cent. before spacing out	Weeks spaced out	Carrier-rate per cent. after spacing out
22	8	2
28	6	7
38.5	6	4.5
28	5	4.5
Huts (previously just over mobilization standard)		
17	8	3
Loose Boxes		
19	7	

CHART A. *Effect of "spacing-out" Barrack Rooms.* Shewing carrier-rate per cent. before "spacing-out"—black columns, and after "spacing-out"—shaded columns.



AYLWIN HUTS.

These are extremely interesting as they belong to the same unit as the second barrack-room shewn above. They remained overcrowded (eight men per hut) all through the same six weeks, during which the barrack-rooms were first spaced out and in which the barrack-room population showed a decrease of carrier-rate from 28 to 7 per cent.

They showed a carrier-rate of 12 per cent. at the beginning of this six weeks (October to mid December), and one of 15 per cent. at the end, and the dangerous distribution of carriers, to which reference has already been made: in other words, whilst the spaced out barrack-rooms improved so much that they had only a quarter of their first percentage of carriers, the Aylwin huts showed a percentage raised from 12 to 15.

The Aylwin huts were then spaced out by removing three men from each, leaving five men, who would, thus, each have 41 square feet floor space and 272 cubic feet air space.

It is impossible to say how far the beds were apart, as each Aylwin hut is a law unto itself.

After this arrangement had been in force five weeks, they were again examined and the carrier-rate found to be 6 per cent. Moreover, no hut had more than one carrier in it.

This plan of spacing out the beds to a distance of two and a half feet between each has been carried out in a very thorough way at one large hutment camp with most excellent results up to the present, but I am anxious to get through the dangerous months of the winter and early spring before speaking with too great confidence. I am told, however, that the general health of the camp has improved immensely. The spacing out, of course, tends to diminish all catarrhal diseases.

For the success of such a scheme everything depends on the interest and co-operation of the Officers of the unit. A barrack-room may be spaced out, and in any case the men will get the benefit of additional air-space, but the full benefit will not be obtained if men are allowed to move their beds close together for warmth or company. In one unit, which had a great success in reducing its carrier-rate, scale plans were prepared and the exact position of each bed drawn.

This enthusiastic unit, unfortunately, and for no fault of its own, furnished an example of the return of the carrier-rate to a higher level when overcrowding became again prevalent. Examining the men in the same rooms, it was found that with a total of 593 men in the barracks

(which just allowed a proper spacing out) these rooms showed a carrier-rate of 4.5 per cent.: after six weeks of gradually increasing overcrowding (the total rising to 803 at the date of the second swabbing), the carrier-rate had risen to 11 per cent.

In these short notes I have not dealt with the question of ventilation, not because it is not equally important with spacing out, but because its importance is much more generally realized, and because many recent War Office Orders have dealt with it.

It must be realized, however, that in many camps and barracks, the "reduced lighting" orders have been dealt with in ways which most seriously interfere with the ventilation of both barrack-rooms and huts, and this accounts for much of the increase in the military carrier-rate, as well as of that in the incidence of pulmonary tubercle.

I should again point out that all carrier-rates given in the preceding figures include only those men carrying meningococci which agglutinate with one or other of the four standard sera issued by the Central Cerebro-spinal Fever Laboratory.

SUMMARY.

1. A high meningococcus carrier-rate in a military unit denotes that dangerous overcrowding exists in this unit.

2. Severe overcrowding (i.e. when beds are less than 1 foot apart) is usually accompanied by a carrier-rate (serological) of at least 20 per cent.

(Twenty per cent. is the danger line indicated in the War Office Memorandum on Cerebro-spinal Fever, March 1917.)

3. A carrier-rate of this height will usually imply that the mobilization standard of 40 square feet has been infringed, and also that beds are less than 1 foot apart.

4. A carrier-rate of 20 per cent. (without awaiting the occurrence of any actual cases of the disease) should be regarded as a signal for prompt and effective action to abolish overcrowding, and to improve ventilation, and to increase the distance between the beds to at least $2\frac{1}{2}$ feet.

5. The distance between beds is of paramount importance.

6. Carrier-rates of between 10 and 20 per cent. are unsatisfactory and imply a certain degree of overcrowding. They must be watched with suspicion, and the mobilization standard strictly enforced.

7. Under the same conditions of overcrowding "non-contact" carrier-rates are the same as "contact" carrier-rates.

8. Quite a moderate degree of "spacing out" of beds, combined with simple methods for improving ventilation are highly efficient agents in reducing high carrier-rates.

9. When, however, a unit shows a high carrier-rate, insistence on the restoration of the mobilization standard is not sufficient.

"Spacing out" must be carried further; and a distance of at least 2½ feet between beds insisted on.

The "peace" standard of 3 feet between beds and 60 square feet of floor space with 600 cubic feet of air space would, of course, be still more effective.

10. The mobilization standard, introduced for a grave emergency, is the lowest possible concession to military necessity, which can be allowed with safety.

The charts B and C shew the occurrence of cross infection from bed to bed, in barrack-room and hut respectively.

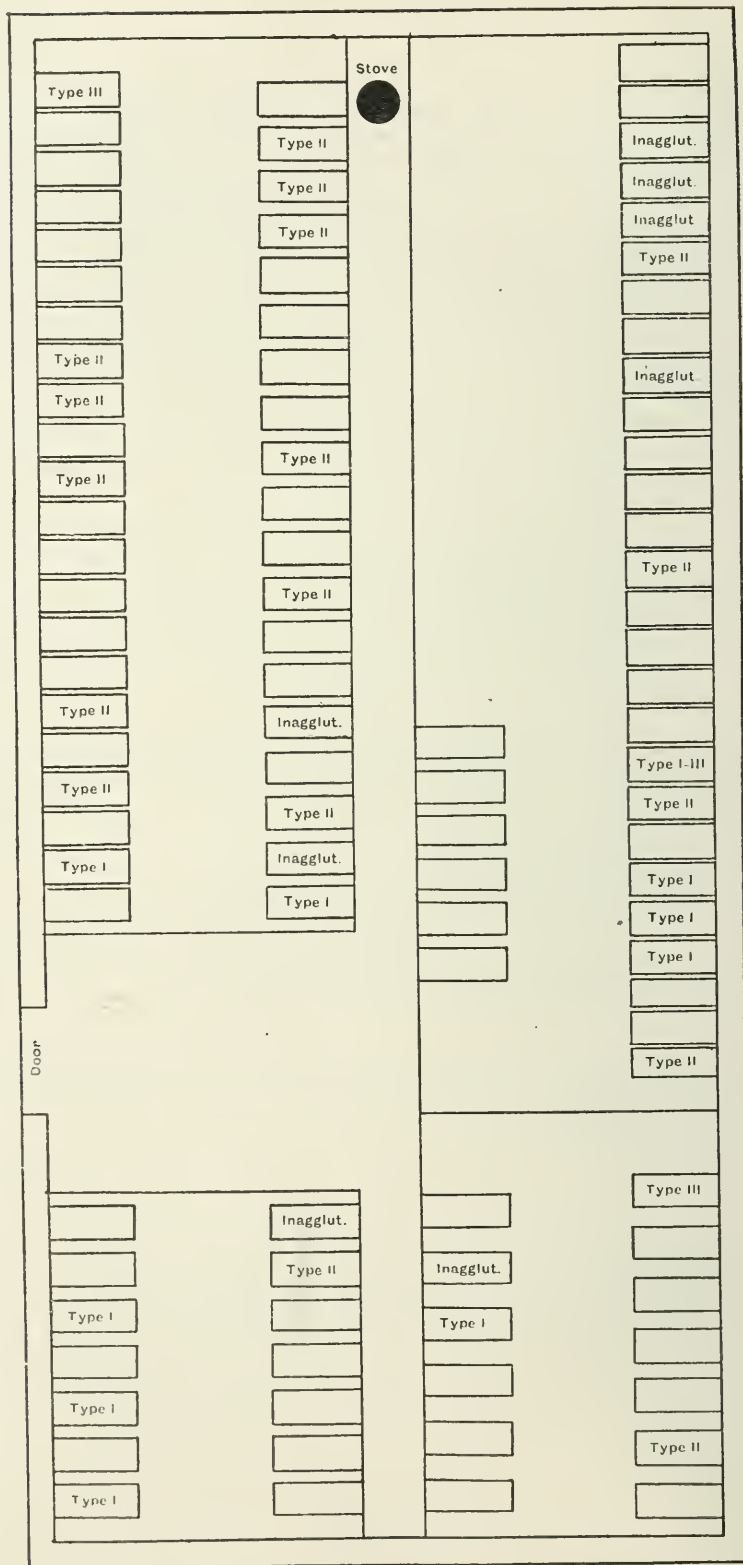
In the barrack-room B, which was adapted from a brewery vat-room, although the floor space and cubic space appear ample yet, owing to fixtures and obstructions, the beds were placed within 9 inches of each other.

Windows were plentiful but of such a pattern that it was impossible to open them without a fearful down-draught, and they were, doubtless, kept shut by night.

The heating apparatus consists of one poor stove at one end, and the cold resultant would contribute to this shutting of windows.

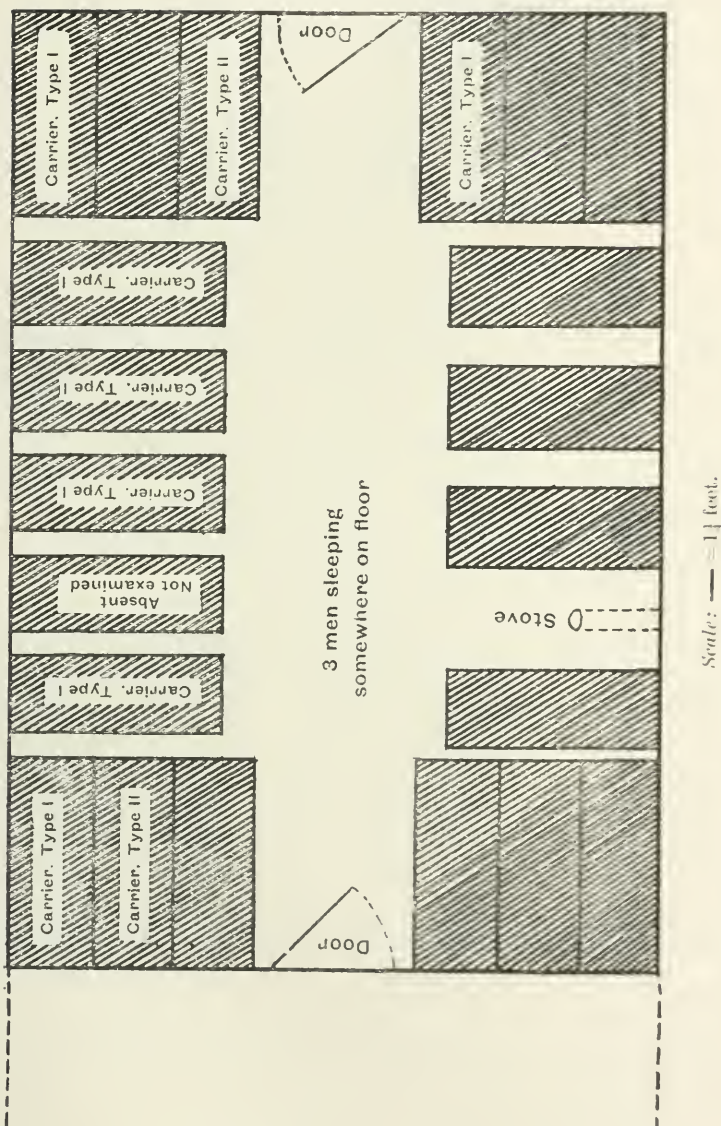
The groups of carriers of Type I meningococcus, of Type II meningococcus, and of organisms resembling meningococci, which do not agglutinate, in adjacent beds or close to each other in corners, are very striking.

Remarkable improvement followed spacing out, and the fixation of some of the windows, which were converted by a simple method into hopper-side ventilators.

CHART B. *Cross Infection due to proximity of Beds.*

This large Barrack-room was adapted from a Brewery Vat-room. Much of the floor space was not available for sleeping owing to obstructions, etc. Plenty of windows, but owing to their make causing intolerable down-draughts these were not opened at night. Heating by one totally inadequate stove. Note the grouping of carriers of the same type of meningococcus particularly in corners and near the stove.

CHART C. *Cross-infection due to overcrowding.* Plan of half an overcrowded hut which was divided by a complete partition (with a door) into two halves each $30' \times 20'$ —showing the distribution of carriers.



In addition to the bed boards shewn, all of which were occupied, three men slept on the floor.

Each man had, therefore, only 25 sq. ft. of floor space.

Owing to the doors, the beds on the short sides are actually in contact. The hut was of superior type to the ordinary army pattern and the windows were of a different pattern.

A CONTRIBUTION TO THE STUDY OF MENINGOCOCCI.

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(A Report to the Medical Research Committee.)

(With 5 Charts.)

It has been shown by numerous observers that well-marked serological differences are found between different strains of meningococci. And since the work of Dopter on the relation of "parameningococci" to meningococci and to cerebro-spinal fever, there has arisen a fairly general agreement that among meningococci capable of causing cerebro-spinal meningitis two broadly separable groups can be defined by immunological methods. But beyond this point considerable divergences of opinion appear. On the one hand there are a number of recent workers who have failed to convince themselves that the two groups of meningococci are in reality clearly delimited, permanent and independent entities. On the other hand certain other workers claim to have still further subdivided these micro-organisms into four definite and independent types, each of which possesses in itself the value of a bacterial species [cp. Andrewes (1917)].

The extended investigations carried out by Dr A. Eastwood, Dr Fred Griffith, and Dr W. M. Scott, for the Medical Officer of the Local Government Board (1917), have led them to the conclusion that any attempt to establish further subdivisions among meningococci, beyond the broad division into the two groups already mentioned, would necessitate the recognition of a large and uncertain number of ill-defined sub-groups. These sub-groups would lack individuality. They would fail to be clearly and decidedly marked off from one another, but would merge gradually one into another by means of intermediate varieties. Moreover even the two main groups themselves seem to be linked by less determinate strains whose serological reactions exhibit intermediate characters.

On the other hand Lieut.-Colonel M. H. Gordon (1917) and his co-workers at the Central Cerebro-Spinal Fever Laboratory have obtained results in their investigations which they interpret as evidence for the existence of four specifically different types of meningococcus. And though their Types I and II and their Types II and IV are described as being somewhat closely allied, each of these serologically distinguishable types is provisionally held to constitute a definite bacterial species. As the result of a very laborious and extended inquiry carried out in the study of these four "epidemic types," it is claimed that substantially all meningococci capable of producing epidemic cerebro-spinal meningitis among troops belong to one or other of these four specifically different types of organism.

Now the question whether the causal agent of cerebro-spinal fever is bacteriologically one or two, or four different organisms is clearly one of fundamental importance as a scientific problem. Its practical bearing acquires significance in relation to treatment, particularly as regards sero-therapeutic measures.

In the course of certain experiments upon the agglutination of meningococci, my attention was more particularly directed to this aspect of the subject by results obtained by Major A. G. Gibson and Mrs Ludlow Hewitt. During their investigation of meningococci recovered from the cerebro-spinal fluid of cases of cerebro-spinal fever they found:

(1) That when meningococci, which on first isolation had fallen more or less readily under one or other type, were employed in the immunisation of rabbits, they gave rise to the development of agglutinating serums whose specificity of type was often very much less clearly defined.

(2) That on inoculation in the human subject two particular types of meningococci, which had been similarly defined, gave rise to the development in the serums of the individuals thus immunised of agglutinins *for different types and in one instance for the different type only*, within the limits of their observations.

Mrs Hewitt and Major Gibson give an account of their investigations in an accompanying Report. Results such as they record bring very sharply into consideration the question how far serological differences of a particular character defined in relation to the serum of immunised rabbits, between micro-organisms which are morphologically and culturally indistinguishable, have any necessary or assured application to the human subject. They suggest that, so far at any rate as concerns disease in man, criteria of differentiation which may possibly hold in

relation to the rabbit, but have not at present been proved to hold for man, may perhaps not possess any primary or fundamental importance in relation to human infection. This aspect of the question appears to acquire additional weight not only from the absence of any notable differences in pathogenicity among the four types, but also from the fact, referred to several times by Lieut.-Colonel Gordon himself, that when a different animal—the horse—is made use of in the preparation of immune serums, the agglutinins produced show *much less specificity* of type than seems to be the case with those of the rabbit.

That this statement of Lieut.-Colonel Gordon represents a moderate presentation of the facts is shown by the results obtained on testing Lister Institute Therapeutic type serums of the horse against Central C.S.F. Laboratory emulsions of type cocci (Table I). For it becomes clear that in some cases, at any rate, the phrase “much less specificity” would be better replaced by the words “complete change of type.”

TABLE I.

Agglutination tests of Lister Institute Therapeutic Anti-Meningococcus Serums against Central C.S.F. Laboratory Emulsions.

	Coccus	25	50	100	200	400	800	1600	3200	Control
Serum	Type I, Batch E	0	0	0	0	0	0	0	0	0
Type I	Type II, Batch E	0	0	0	0	0	0	0	0	0
Lister Insti-	Type III, Batch D	0	0	0	0	0	0	0	0	0
tute 58 D.	Type IV, Batch C	<i>t</i>	<i>t</i> -	<i>p</i> +	<i>p</i> -	0	0	0	0	0
Serum	Type I, Batch F	<i>t</i>	<i>t</i> -	<i>p</i> -	<i>tr</i> -	? <i>tr</i>	0	0	0	0
Type III	Type II, Batch D	0	0	0	0	0	0	0	0	0
Lister Insti-	Type III, Batch D	? <i>tr</i>	0	0	0	0	0	0	0	0
tute 57 D.	Type IV, Batch C	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i> -	<i>tr</i>	0	0	0	0

t = total agglutination, *p* = partial, *tr* = trace. The exact notation used is described on p. 395.

But even as regards the rabbit, it may fairly be urged on the information at present available, that the evidence put forward in favour of the specific character of the serological differences between the four types of meningococci remains inconclusive. And for the reasons which will be discussed immediately, the observations and experiments on which reliance has been placed by Lieut.-Colonel Gordon for the specific differentiation of his four types may still be regarded as at any rate indecisive.

Before any so far-reaching a conclusion should receive assent, or be accepted as a final basis for therapeutic measures, it ought to be established on the most secure foundations. Until this has been achieved

the view that there are four clinically identical, but bacteriologically distinct epidemic cerebro-spinal fevers must be received with a good deal of caution. Undoubtedly it is the case that were this proved, a sufficiently striking parallel exists in the three bacteriologically distinct enteric fevers. But in that connection it is necessary to note that while recovery from infection with any one of the three typhoid-paratyphoid organisms leaves the individual susceptible to infection (even immediate infection) with either of the other two, one does not see or hear of second attacks of cerebro-spinal fever. A single proved instance of a second attack of meningitis due to a type of meningococcus different from that which caused the first attack would afford very weighty evidence in support of the theory of specificity of type. But until such evidence may be forthcoming it would appear that an individual who recovers from cerebro-spinal fever has probably become immune to all four types.

In suggesting the propriety of an attitude of doubt and hesitation in regard to the alleged bacteriological specificity of the four types of meningococci, it is important to state clearly that it is not thereby intended for a moment to imply any doubt regarding the very great value of the practical measures which have been introduced and so successfully carried out in relation to cerebro-spinal fever among troops. For it cannot fail to be universally recognised that these measures have achieved remarkable success in the detection and isolation of infected persons, in the limitation of epidemic outbreaks, and in the recognition of the source and origin of particular meningococcal infections, as well as in the actual treatment of the disease.

The only question which is raised at the moment is the question whether the evidence is at present adequate to justify the claim put forward. Namely that it "would appear to indicate in no uncertain manner that the four types of meningococcus...are stable entities, that they are specifically distinct from one another," and that the cases of cerebro-spinal fever investigated were due "not to transient and unstable variants of a single micro-organism, but to a group of individual species of meningococcus." The definition of types serologically different in their reaction with the immune serums of rabbits, though otherwise similar, may afford information of great practical utility, in particular circumstances. But it does not necessarily afford an evidence that the differences in question are specific unless those serological reactions are constant. It is moreover of relatively little importance how many strains, or how great a percentage of all strains investigated fall into the classes proposed, if instances are found in which some strains so

placed subsequently exhibit variation of type, and refuse to remain in their original class. A few such instances would suffice to prove that the classification lacks specific value.

THE QUESTION OF FIXITY OF TYPE.

It is therefore clearly fundamental to the theory of specificity of type in the four groups of meningococcus, that these types should be demonstrably "fixed" and in no circumstances interchangeable.

Upon this point Lieut.-Colonel Gordon records the fact that neither under prolonged sub-cultivation, nor otherwise, has he met with change of type or gross variation in the very numerous meningococcal cultures which have come under his observation. In agreement with this statement most other workers appear to have found a considerable degree of constancy in their strains; though differences in agglutinability in different subcultures has quite frequently been marked, and differences of degree in sugar fermentation have often been noted. But while, as might be expected, this is undoubtedly the general trend of recorded observations, a study of the literature at once brings out the fact that the majority of recent workers have drawn attention either to a single isolated instance, or sometimes to several cases, in which particular strains of meningococcus failed to maintain fixity of type during the period of observation, or even at one and the same moment belonged equally to two different types.

A few examples may be quoted in illustration:

1. Gordon and Murray (1917) have referred to the occurrence of an "amphoteric" strain—Types I and III, which absorbed the specific agglutinin of both these types.

2. Arkwright (1915) found a strain which agglutinated in both his groups, and another which changed serologically from one group to the other during the period of observation.

3. W. M. Scott (1917) has derived two different strains from a particular culture, the one possessing the serological characters of his Group 1, the other the serological characters of his Group 2.

4. Walker, Hall and Peters (1916) found in two cases that the serological reactions of the meningococci isolated from the cerebro-spinal fluid of a patient on successive days differed in type.

5. On two occasions I have had the opportunity of examining the serum of two human subjects who had been repeatedly inoculated by Major A. G. Gibson with Type I and Type III meningococcus respectively. On each occasion agglutinins were present in small amount for

Type III and Type IV meningococcus respectively, but not for any other type (the tests being made against emulsions issued from the Central C.S.F. Laboratory). That is to say a Type I antigen led to the production of Type III agglutinins, and a Type III antigen to the production of Type IV agglutinins.

Fixity of type—that is to say the existence of different species among meningococci—could therefore only be maintained on the assumption that all the observers here referred to had fallen into error, and were mistaken in their observations. And the more extensive the record of such observations becomes, the greater is the probability that the differences which exist between the four types are insufficient to amount to differences of species. It is, therefore, of importance that among 356 strains of meningococci W. J. Tulloch found no less than 23 (6·5 per cent.) which could not be placed either as specifically type cocci, or even as cocci showing the common group relationship of Types I–III, or Types II–IV by agglutination tests; and three out of the 107 to which the absorption test was also applied (2·8 per cent.) which still failed to qualify in respect of specificity in relation to rabbit serum.

THE METHOD OF “SATURATION.”

The most weighty experimental evidence adduced by Lieut.-Colonel Gordon in favour of the specificity of the four types has been derived from two series of investigations, the first carried out by Major Hine and himself, the second by Major Hine and Captain Tulloch.

In the first of these, immunity tests were made on animals immunised with one or other of the four types of meningococcus by determining in each case the fatal dose of a particular type when the animal was treated by the “saturation” method of Gordon and Horder (1907–8). The results so obtained are regarded as lending support, so far as they go, to the view that the protection obtained by immunisation with type meningococci is of a *univalent* character. The inference drawn is that they help to justify the claim for the specificity of the types. But a consideration of the data presented seems to lend weight to a contrary opinion concerning the validity of this inference.

The two experiments under discussion are quoted in Table II below where the two series of results have been placed side by side for ease of comparison.

In each experiment four rabbits were immunised against each of the four types of meningococcus. The animals thus immunised are represented in four horizontal rows in the table, one row for each type

of coccus. In each experiment one rabbit died during immunisation. Ten days after the last immunising dose the animals, along with suitable control rabbits (represented in the lowest row), were "saturated" with living meningococci of appropriate type in correspondence with the vertical columns of the table. The procedure was carried out by injecting into a vein of the animal the living cocci from one slope culture of the type concerned every hour until death occurred, or until all the animals but one in any given column had succumbed.

The numerals indicate the number of hourly intravenous inoculations given, the sign minus (−) signifies that the animal dies, the plus (+) sign that it survived the last dose administered. The bracketed figure indicates that though the animal concerned did not die it became ill and remained so for some time.

TABLE II.
Gordon and Hine's "Saturation" Tests.

Protecting Coccus	Saturating Coccus							
	Type 1		Type 2		Type 3		Type 4	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Type I	−6	−2	−12	−8	−5	(+8)	...	−9
Type II	−8	−8	+12	+8	−5	−8	−9	−9
Type III	−8	−6	−12	...	+8	+8	−9	−9
Type IV	−3	−8	−8	−4	−8	−7	+9	+9
Control	−8	−6	−6	−6	−8	−7	−8	−8

The following considerations appear to be relevant in regard to these results:

1. The critical margin of dosage between animals that lived and those that died is a very small one upon which to base a far-reaching conclusion. It amounts to no more than one slope culture in a total of 8, 9 or 12; that is to say from 8 per cent. to 12 per cent. of the total dosage. A difference of this magnitude would fail to carry conviction even had the content of the doses been more accurately measured, and the weights of the animals recorded in relation to the dosage. And it is evident that doses reckoned only in "slope cultures" are open to variations which might easily outweigh this slight margin.

In the animals saturated with Type III coccus the two controls show a difference of one hour and one slope culture in the fatal result. Yet a conclusion is based on a difference of only this extent between homologously and heterologously immunised animals. And under Type I there is a range of fatal dose for the controls of two slope cultures.

It is therefore much to be regretted that experiments of so laborious and protracted a character as these undoubtedly are, were not rendered more convincing by continuing the inoculations in the animals which survived to a point which might have placed the results entirely beyond question, had it been reached successfully. For as the matter stands it is legitimate to suggest that some or all of the surviving rabbits might have succumbed to the next inoculation.

2. If the four types of cocci are in reality specifically different, and if, as is claimed, the immunity conferred is therefore "mainly of a univalent character," the *heterologously* immunised rabbits should fail to show evidence of any significant degree of protection. This is held to be the case.

It will, however, be observed that many of these animals easily outlived their controls, as shown in the accompanying table (Table III).

TABLE III.

Compiled from Gordon and Hine's Saturation Tests.

Saturating coccus	Heterologously immunised rabbits which outlived their control animals		Number of slope cultures by which they outlived the controls
	Number	Percentage	
Type I	2 out of 6	33	2
Type II	2 out of 5	40	6
"	4 out of 5	80	2 or more
Type III	2 out of 6	33	1
Type IV	5 out of 5	100	1

The experiments in fact afford much more convincing evidence of some degree of general protection of the heterologous animals, than any which they offer in support of a specific univalent immunity in the homologously immunised individuals. For the latter in no case amounts to more than one slope culture, while in one experiment two individuals out of three survive until they have received a dose which is *double* that which killed the corresponding control.

3. In other directions also the experiments appear to prove too much or too little for the security of the view which its authors are at present inclined to maintain. For if immunisation with heterologous meningococci were in the main indifferent as regards protection, the animals thus treated *should behave like control animals* when submitted to the test by "saturation." But this is not the case as can be seen by comparing the range of the fatal dose in the two series (Table IV).

The heterologously immunised rabbits show a wide range of fatal dose with three of the types, namely 300 per cent., 200 per cent. and

60 per cent. respectively; while the greatest variation in fatal dose for control rabbits is 33 per cent.

TABLE IV.

Compiled from Gordon and Hine's Saturation Tests.

Rabbits	Fatal dose expressed in slope cultures, for control rabbits and for the heterologously immunised rabbits			
	Saturating Coccus			
	Type I	Type II	Type III	Type IV
Control	6 to 8	6	7 to 8	8
Immunised	2 to 8	4 to 12	5 to 8	9

Only two possible explanations suggest themselves: either (1) that the true range of fatal dosage for unprotected animals possessed the wide limits shown by the heterologously immunised rabbits (on the supposition that they were really unprotected as is claimed). This is improbable, but if true it would at once deprive the whole of the observations of the significance intended. Or (2) that some of the immunised animals under discussion were in fact moderately protected by their heterologous immunisation, as already suggested (i.e. the types are not *specifically* different); while others, like the two homologously immunised Type I rabbits (see Table II) had actually become more susceptible than normal.

As shown in Table V no less than 4 rabbits out of 15 (26·7 per cent.) in each experiment succumbed to doses less (often much less) than those required to kill their control animals. An explanation of this fact should be forthcoming. It may be sought along the following lines.

TABLE V.

Compiled from Gordon and Hine's Saturation Test.

Saturating coccus	Heterologously immunised rabbits which died before their control rabbits		Number of slope cultures by which their controls out-lived them
	Number	Percentage	
Type I	2 out of 6	33	4 to 5
Type II	1 out of 5	20	2
Type III	2 out of 6	33	3
Type IV	0 out of 5	3	—

4. At the time when the "saturation" method of testing virulence in meningococcus was introduced by Gordon and Horder, these authors showed that while after a single massive intravenous inoculation cocci could be cultivated from the peripheral circulation for 12 hours or more, they could never be grown from the blood when the "saturation" method was used. They also found that in the latter case a marked leucopenia

appeared, instead of the usual leucocytosis. But neither then nor in the present connection have data been recorded regarding the manner of death of these animals and the post-mortem findings.

The possibility at once presents itself to the mind that these animals (or some of them) when inoculated hourly with one whole slope culture intravenously, die from mechanical causes, rather than from bacterial intoxication. It was shown many years ago by Jörgensen and Madsen that the introduction of massive doses of *B. typhosus* intravenously in animals possessing high titre agglutinating serums might lead to death by the production of multiple embolisms. And it is of importance to observe that the two homologously immunised animals which died early in the experiments now under consideration were the Type I rabbits. The interest of this observation lies in the fact, noted by Gordon, that Type I meningococcus "is perhaps the best type of all for exciting the production of agglutinin by the rabbit."

In view of these facts I have devoted some attention to the "Saturation" method, and have found with three different micro-organisms, one of them *non-pathogenetic*, that the immediate cause of death in the cases hitherto observed has appeared to be a *widespread thrombosis of capillaries and veins in the pulmonary circulation*. In the course of these observations three rabbits have been killed by the method in question; the first with *Meningococcus*, the second with *B. typhosus*, and the third with *Sarcina lutea*. In each case a careful post-mortem examination was made, and numerous microscopical sections prepared from the various organs. The whole material was subsequently handed over to my friend Major A. G. Gibson who has kindly consented to append a Report on the histological appearances.

In the experiments of Gordon and Hine the number of meningococci introduced in the more prolonged tests was probably of the same order of magnitude as the total number of red corpuscles in the blood, and almost certainly many times greater than the total number of leucocytes in the circulation. In the third of my own experiments the total number of cocci (*Sarcina*) introduced was doubtless still greater.

PROTOCOLS OF SATURATION EXPERIMENTS.

In each case a large number of slope cultures was grown for twenty-four hours. They were then carefully emulsified in normal saline solution, mixed together to secure uniformity of dosage, and made up to such a volume that 1 c.c. represented one slope culture (or in the case of *Sarcina* 1.1 slope culture and 1.5 slope culture on two different occasions).

Experiment 1. Meningococcus.

A healthy male rabbit of 2500 grammes weight, which had been under immunisation with meningococci for seventeen days, but received its last inoculation nine days before the day of saturation, and had been gaining weight during the whole period of observation, was saturated by means of hourly intravenous injections of 1 c.c. (one slope) of emulsified living meningococci.

The animal was obviously in distress after eight inoculations. After the tenth it was helpless and in a dying condition. No further inoculation was given. The animal died between the eleventh and twelfth hour.

At the post-mortem examination there was marked patchy congestion of the lungs with sub-pleural haemorrhages. There was one sub-peritoneal haemorrhage in the small intestine; marked congestion and a number of small haemorrhages in the vermiform appendix.

Microscopically the immediate cause of death was extensive pulmonary thrombosis.

Experiment 2. B. typhosus.

A healthy female rabbit of 2250 grammes weight was saturated by means of hourly intravenous injections of 1 c.c. (one slope) of emulsified living *B. typhosus*.

A few minutes after the third inoculation convulsive struggling occurred followed by extensor spasm, and the animal died within a minute or two.

An immediate post-mortem examination was made with due precautions. There was a serous effusion in the pericardial sac, a marked patchy congestion of the lungs, a small sub-epicardial haemorrhage, and a small area of sub-capsular haemorrhage in the liver. The cause of death was widespread thrombosis of vessels in the lungs.

Experiment 3. Sarcina lutea.

A healthy male rabbit of 2850 grammes weight was saturated by means of hourly intravenous injections of 1 c.c. (1·1 slope) of emulsified living *Sarcina*. Eight doses were given without producing any recognisable effect whatever, except that almost immediate clotting occurred in the vein at the site of each successive inoculation. The animal's weight fell 50 grammes for one day only, and it appeared to remain in perfect health.

Ten days later, its serum then showing evidence of some agglutinating action on a formalised bouillon culture of *Sarcina*, it was again saturated,

receiving intravenous inoculations of 1 c.c. (1·5 slope) of emulsified living *Sarcina* hourly for three doses, and then at intervals of three quarters of an hour until the tenth dose. On this occasion it was observed that no clotting occurred in the veins at the sites of inoculation, in striking contrast to the result seen on the earlier occasion.

From the seventh inoculation onwards the condition of the animal began to fail; and thirty-five minutes after the tenth inoculation it fell into convulsions, which passed on into a rigid extensor spasm, death occurring in about five minutes.

An immediate post-mortem was made. There was serous effusion in the pericardium, and extreme patchy congestion with oedema of the lungs.

The cause of death was extensive thrombosis of the pulmonary capillaries and veins.

In none of the foregoing experiments were blood cultures made from the peripheral circulation. But in each case a number of films of blood from a peripheral vein were examined. No organisms could be found in any of these films, except for a single small group of half a dozen *Sarcina* seen in one of the films from the third experiment. But examination of the histological sections at once suggests an explanation both of the leucopenia and of the absence of cocci in the peripheral blood noted by Gordon and Horder. For there is an enormous accumulation of leucocytes and micro-organisms in the capillaries and veins of internal organs; particularly the lungs, which constitute a first filter for the micro-organisms injected into the peripheral veins, and eventually become obstructed by the extending thrombosis. Accordingly it seems not unreasonable to suggest that the comparatively early occurrence of pulmonary obstruction and thrombosis affords a probable explanation of the fact that so many of the immunised rabbits in Gordon's and Hine's experiments died before the control animals. Possibly it is a common cause of death in animals treated by the "saturation" method; and it may be the usual cause. But if this is so the results obtained in such experiments require to be interpreted with extreme caution. In any case it is a fair supposition that we may thus account for the death of those rabbits which died *earlier* than any one of the eight control rabbits. If therefore we omit these individuals from consideration for the moment, and calculate the average death time for all other heterologously immunised animals, and the average death time for the eight controls an interesting result emerges. For this purpose I take the lethal dose for the first animal in column 6 as 9 slope cultures, since this animal did

not die with a dose of 8 although it became ill. The lethal dose at any rate could not have been *less* than 9 slope cultures, though it might have been more.

On making the calculation just referred to it appears that while the average lethal dosage for control rabbits is 7.1 slope cultures, that for the remaining heterologously immunised rabbits is 8.6 slope cultures. That is to say the fatal dose for the latter is 21 per cent. greater than that for the controls. The margin is not large, but so far as it goes it is in opposition to the view that the immunity conferred by type meningococci is monovalent. It is at any rate a greater margin of dosage than that upon which Gordon's conclusion was founded (about double), since the latter never exceeded 8 to 12 per cent. of the total dosage.

SUPERIMPOSITION TESTS.

Under this term Lieut.-Colonel Gordon records experiments in *successive immunisation* with different types of meningococci. The experiments were carried out by Major Hine and Captain Tulloch. Only the first of them is given in any detail in the Report. In this experiment five rabbits were immunised with Type I coccus as primary antigen. When their blood had acquired agglutinating power for Type I they were inoculated with a second antigen as shown in the table below (Table VI).

The records demonstrate the fact that each secondary antigen produced agglutinins for itself, and that its effect did not (in two cases out of three) prevent the steady fall of the agglutinins for Type I which were being formed as the result of the earlier inoculations with Type I. In the third case a marked secondary rise of the Type I agglutinins to at least double their previous maximum titre was induced.

The conclusion drawn is that the agglutinins for the different types are *entirely* independent; and the inference is that the four types are four distinct though to some extent allied species.

The first point of importance in regard to these results is the fact that the titre of agglutinins for the secondary antigens of Types II, III and IV never exceeded a dilution of 1 in 500, yet the lower limit taken for agglutinin readings has been arbitrarily fixed at 1 in 100. Normal rabbit serum, within the limits of my present experience, does not in ordinary circumstances give more than traces of agglutination, if so much, with any of the four types at 1 in 20 dilution, and only infrequently gives complete or nearly complete agglutination at 1 in 10

dilution. Occasionally, however, there may be nearly complete agglutination at 1 in 20 and traces up to 1 in 40. But seeing that one always begins an experiment by measuring accurately the normal agglutinins of the animal before inoculation it follows that there is usually in these observations a range of dilutions of valid significance¹ from 1 in 20 up to 1 in 100; a range as extensive as the range from 1 in 100 to 1 in 500, on which reliance is placed, but one apparently unexplored in Hine and Tulloch's observations.

Nevertheless in *two* different cases where the maximum titre shown for the primary antigen was 1 in 600 and that for the secondary antigen 1 in 500, agglutination was present for another type in a dilution of 1 in 200. In the third case the agglutination titre for another type was such as to give a "slight" reaction at 1 in 100. These reactions are dismissed as being due to "group-agglutinins," a point to which reference will be made immediately. Table VI gives a digest of the experiment of Major Hine and Captain Tulloch.

TABLE VI.

Compiled from Hine and Tulloch's Superimposition Experiment.

Rabbit	Primary Antigen Type	Secondary Antigen Type	Titre for primary Antigen 1 in	Titre for secondary Antigen 1 in	Type II agglutinated 1 in		Type III agglutinated 1 in		Type IV agglutinated 1 in	
					100	200	100	200	100	200
A	I	—	600	—	0	0	0	0	0	0
B	I	I	600	600	0	0	0	0	0	0
C	I	II	600	400	—	—	0	0	slight twice	0
D	I	III	600	500	slight twice	slight once	—	—	0	0
E	I	IV	600	500	some 5 times	some 4 times	0	0	—	—

It thus appears that in some cases "group agglutinins" were present to a titre of from *one-third to one-half* the titre of the "specific" agglutinins.

Now whatever theory be held as to the real meaning of "group agglutinins," it will be admitted at any rate that they probably represent some sort of common factor of the agglutinable properties of the different bacterial cultures concerned with the particular sample of serum employed under the conditions provided. And wherever group agglutinins are found to be present opinion will remain divided as to

¹ For the purposes of investigation of known meningococci, as distinguished from the diagnosis of unknown cocci.

the justifiability or wisdom of distinguishing species among the micro-organisms thus grouped, if they are shown to be otherwise closely related. Where bacteria are clearly distinguishable by other biological characters the phenomenon of their "group-agglutination" by serum specific for only one of them tends to become less frequently observed as technique advances. Thus, for example, in the case of *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B, one has never seen any trace of "group agglutination" in thousands of agglutination tests made with standardised agglutinable cultures of these bacteria. The titre of a serum may be many thousand for T. A or B, as the case may be, without the least trace of agglutination for the other two at 1 in 25 dilution and lower.

The facts regarding group agglutination may perhaps have a different basis in the case of meningococcus, and some other organisms. But until much more conclusive evidence is available, it is clearly permissible to hold the view that in low titre serums (such as those under discussion) with a maximum agglutination titre for the homologous coccus of 1 in 400 or so, agglutination in dilutions of 1 in 20, and 1 in 40 (in marked excess over the normal agglutinating power of the serum of the animal before immunisation), and rising to 1 in 80, 1 in 100 or more is evidence to cause hesitation in concluding that the types are specifically distinct.

By the courtesy of Lieut.-Colonel Gordon and Major Hine I have been supplied with samples of the four type emulsions and serums, as well as with living cultures of the four type cocci. I am indebted to their kindness for the opportunity of bringing my experiments and materials obtained from other workers into their proper relation to their standards. I am also indebted to Major Gibson who was good enough to place all his meningococci of cerebro-spinal origin at my free disposal; and to Dr A. Eastwood by whose kindness I have been enabled to make use of a number of attested meningococcal cultures, and their corresponding serums, sent me by himself and by Dr Fred Griffith and Dr W. M. Scott.

A brief account may now be given of experiments bearing on the meaning of the "superimposition tests" (or successive immunisations) carried out for Lieut.-Colonel Gordon by Major Hine and Capt. Tulloch. And it may here be stated that while all the tests recorded in the present communication have been made by himself, all the more important agglutination results were kindly read for me independently by Dr A. D. Gardner, who was kept in ignorance of the dilutions and particular types before him until the readings had been made and recorded.

After determining the limits of agglutination of the four types with normal rabbit serum the first step was to make cross agglutinations with the four type cocci and the four type serums as issued from the Central C.S.F. Laboratory. The results are given below in Table VII, as read after 24 hours in a water-bath at 55° C. In some experiments the tubes were read again after standing in the cold for a further 24 hours. This carries the titre somewhat higher, but it necessitates bringing into use a very large number of stands and tubes. The emulsions gave very excellent results indeed, in so far that the controls did not begin to show any sign of sedimenting spontaneously until they had stood for 36 to 48 hours following the 24 hours in the water-bath.

The drop method was used with small agglutination tubes, as employed for enteric groups and dysentery agglutinations. Ten drops of emulsion of cocci were always added to 10 drops of appropriately diluted serum in normal saline solution.

The notation used in the readings is as follows:

- t = total or complete agglutination with complete sedimentation.
 $t -$ = total agglutination with fluid almost clear, but sedimentation incomplete.
 p = partial agglutination; marked sedimentation, but considerable opalescence of the fluid remaining.
 $p+$ = more than p , and less than $t -$.
 $p-$ = less than p .
 tr = traces of agglutination; some deposit, and slight clearing of opalescence.
 $tr+$ = larger traces, but less than $p -$.
 $tr-$ = a very slight deposit, and opalescence distinctly less than in the control titre.
 $?tr$ = doubtful.

The cross agglutinations shown in Table VI were carried out with the materials named below obtained from the Central C.S.F. Laboratory.

Serums: Type I, Batch B.	Emulsions: Type I, Batch D.
Type II, Batch B.	Type II, Batch C.
Type III, Batch B.	Type III, Batch B.
Type IV, Batch B.	Type IV, Batch C.

The readings recorded in Table VII show the extent to which agglutination occurred with each type serum against the four type emulsions. With serum Type I and serum Type II coccus Type IV shows no higher

agglutination than might have been found with normal rabbit serum. The same is possibly true of coccus Type II with serums Type III and Type IV. But in a number of cases cocci of Types I, II or III agglutinate with heterologous serums up to a titre from one-twentieth to as much as one-fifth or more of the titre given with the homologous coccus.

TABLE VII.
Cross Agglutinations.

Serum	Emulsion of cocci	Dilution 1 in								Control
		20	40	100	200	400	800	2000	4000	
Type I	Type I	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>tr</i> +	0	0	0
	„ II	<i>t</i>	<i>t</i>	<i>p</i>	<i>tr</i> -	—	—	—	—	0
	„ III	<i>p</i> +	<i>p</i>	0	0	—	—	—	—	0
	„ IV	<i>tr</i> -	0	0	0	—	—	—	—	0
Type II	„ I	<i>t</i>	<i>t</i>	<i>tr</i> -	0	—	—	—	—	0
	„ II	* <i>p</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>p</i>	? <i>tr</i>	0
	„ III	<i>tr</i> +	<i>tr</i>	? <i>tr</i>	0	—	—	—	—	0
	„ IV	<i>t</i> -	<i>p</i> -	<i>tr</i> -	0	—	—	—	—	0
Type III	„ I	* <i>t</i> -	<i>t</i>	<i>t</i>	<i>p</i>	—	—	—	—	0
	„ II	<i>tr</i>	<i>tr</i> -	0	0	—	—	—	—	0
	„ III	* <i>t</i> -	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i> -	<i>tr</i>	0	0	0
	„ IV	? <i>tr</i>	0	0	0	—	—	—	—	0
Type IV	„ I	<i>t</i> -	<i>p</i> +	0	0	—	—	—	—	0
	„ II	<i>t</i> -	<i>tr</i> +	0	0	—	—	—	—	0
	„ III	* <i>t</i> -	<i>t</i> -	<i>tr</i> -	0	—	—	—	—	0
	„ IV	* <i>t</i> -	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	0	0	0

A — signifies that the test concerned was not made.

* = clear evidence of the existence of a zone of inhibition.

Now it is stated that of the four types, Types I and III and Types II and IV are the most nearly allied. But in the present test it is seen that:

(a) Type I serum carried up Type II coccus much more than Type III coccus.

(b) Type II serum carried up Type I coccus at least as much as Type IV coccus.

(c) Type III serum carried up Type I coccus most, and acted little on Type II and very little if at all on Type IV coccus.

(d) Type IV serum carried up Type III coccus most, and acted more on Type I than on Type II coccus.

The Type III serum was, therefore, the only one of this batch which gave results at all suggestive of the relation claimed to exist.

In order to present further data bearing on this question the results of two immunisation experiments may be quoted. In the first of these a male rabbit of 1900 grammes weight was inoculated intravenously with 0.5 c.e. (approximately 1000 million cocci) of the Type I emulsion named above on the *first*, *third* and *fifth* day of experimentation. Agglutination determinations of its serum against Central C.S.F. Laboratory emulsions are given in Table VIII for day 1 (before inoculation) and days 5, 6 and 8. They show a fairly rapid production of agglutinins for Type I, and a synchronous gradual development of increasing, though much less, amounts of agglutinin for all four types.

By day 8—seven days from the first inoculation—the agglutination of the homologous coccus is complete up to 1 in 800 dilution, and partial at 1 in 2000. The titre for Types II and III is pretty nearly equal, reaching 1 in 100. But (for what it is worth) Type II is somewhat more agglutinated than Type III, though earlier on the agglutinins for Type III had developed more rapidly than those for Type II. Type IV agglutinins are laggard and very feeble, as was also the case in two instances in Table VI.

TABLE VIII.

Immunisation with Type I Coccus. Agglutination Determinations.

Coccus Dilution 1 in	Type I										Type II					
	10	20	40	80	100	200	400	800	2000	Control	10	20	40	80	100	Control
Day 1	<i>tr</i> +	? <i>tr</i>	0	0	0	—	—	—	—	0	? <i>tr</i>	0	0	0	0	0
Day 5	<i>t</i>	<i>t</i> -	<i>p</i> +	<i>tr</i>	0	—	—	—	—	0	? <i>tr</i>	0	0	0	0	0
Day 6	<i>t</i>	<i>t</i>	<i>t</i> -	<i>p</i>	<i>tr</i> +	<i>tr</i>	0	—	—	0	<i>t</i> -	<i>tr</i>	0	0	0	0
Day 8	* <i>tr</i> -	* <i>t</i> -	* <i>t</i> -	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i> -		<i>p</i>	0	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i> -	<i>p</i> -	0

Coccus Dilution 1 in	Type III							Type IV			
	10	20	40	80	100	Control	10	20	40	Control	
Day 1	<i>tr</i>	? <i>tr</i>	0	0	0	0	0	0	0	0	
Day 5	<i>p</i>	<i>p</i> -	<i>tr</i>	? <i>tr</i>	0	0	<i>p</i>	<i>tr</i>	0	0	
Day 6	<i>t</i>	<i>t</i> -	<i>tr</i>	? <i>tr</i>	0	0	<i>p</i> +	<i>tr</i>	0	0	
Day 8	* <i>p</i>	* <i>t</i> -	<i>t</i>	<i>p</i>	<i>tr</i> +	0	<i>t</i> -	<i>p</i> -	0	0	

— signifies test not made.

* indicates clear evidence of a zone of inhibition.

In the second experiment a male rabbit of 2450 grammes weight was inoculated on the *first*, *third*, and *fifth* day of experimentation with 0.5 c.e. (approximately 1000 million) of the Type II emulsion, and on the *ninth* day with 1 c.e. (approximately 2000 million) of the Type III emulsion. Agglutination determinations against Central C.S.F. Laboratory emulsions are given in Chart I for day 1 (before inoculation) and for days 7, 9, 12, 14 and 16.

The Chart illustrates the course of the development of agglutinins for all four types during successive immunisation with two of the types.

Many points of interest appear and among them may be noted the comparative ease with which the titre of agglutination for the homologous coccus can be made to rise.

Keeping the foregoing evidence in view, the "superimposition tests" may now be considered in somewhat greater detail. And it now seems probable that the appearance given in Lieut.-Colonel Gordon's charts

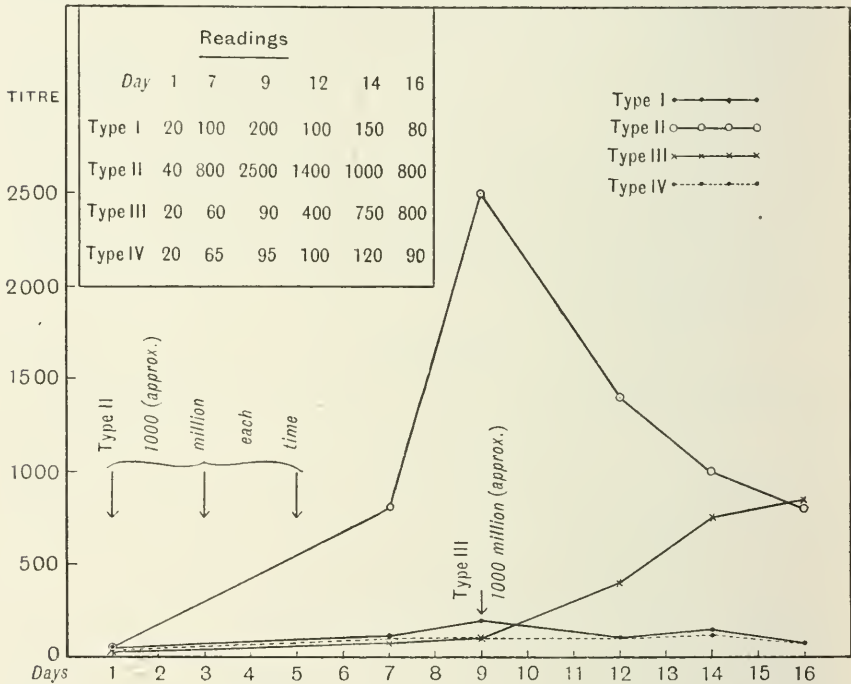


Chart I. Agglutination Titre of Serum of Rabbit for the four Types of Meningococcus during successive immunisation with two of the Types.

of clear and sharply defined *qualitative* differences in the agglutinin response to inoculations with different types of the meningococcus is in reality due to the omission of all measurements of titre below the 1 in 100 dilution. Though even so, agglutination for types other than those used for inoculation was frequently detected at 1 in 100, and sometimes also at 1 in 200 dilution, that is to say, up to one half or nearly half the titre found for the second antigenic type.

Nevertheless, it might perhaps remain a matter of doubt whether these results, along with the results of the Absorption tests, to which

decisive value is attached by Lieut.-Colonel Gordon, did not entitle the four types of meningococcus to be ranked as "independent entities" were no collateral evidence available.

But if it is shown that a series of different strains of *B. typhosus*, for example, may sometimes yield precisely similar results, the interpretation to be placed on evidence of this character will become much clearer. For presumably no bacteriologist will suggest that different strains of *B. typhosus* constitute different bacterial species.

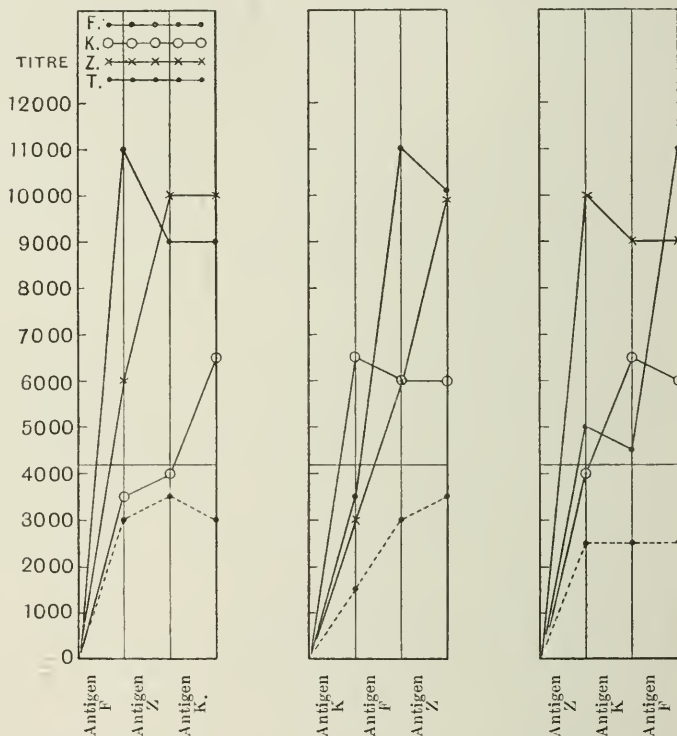
The "superimposition tests" are in principle closely similar to experiments in successive immunisation carried out by myself (1901) with different strains of *B. typhosus* in the Swiss Serum Institute at Berne (1899-1900). In the experiments material to the present discussions four strains of *B. typhosus* were employed, denoted F, K, Z, and T and rabbits were immunised successively with three of the strains (F, K, Z) in different orders of succession. At the end of each stage, or period of immunisation the serum of each rabbit was tested to determine its titre of agglutination against each of the four strains.

The results obtained are shown in Charts II, III and IV, which have been re-drawn from my original paper. They appear to me to show in greater or less degree all the same points as are emphasised by Lieut.-Colonel Gordon in the charts obtained by Major Hine and Capt. Tulloch. And if one chose to draw a line across the charts and to ignore agglutination at 1 in 4000 (*about one-third of the maximum titre obtained*) and all below, very misleading conclusions might be drawn from the remaining readings. For example, the four strains might be regarded as specifically independent organisms, of which Types F and Z showed some affinity to each other, while Type K, and to an even greater extent Type T, were more widely differentiated by the serological reaction of agglutination. And it may be noted particularly that there is frequently shown a fall in titre for an earlier antigen, while the titre for the most recently inoculated strain is rising.

But since the experiments referred to are now rather old, and were carried out before the introduction of the present accurately standardized methods, I have recently repeated them with the four strains of *B. typhosus* denoted below as T.E., T.L., T.O., and T.T. These four strains were plated out and carried on from single colonies. They were put through the appropriate tests, and were shown by them and by agglutination tests to be genuine *B. typhosus*. Agglutinable cultures of standardized and equal opacity were prepared from the strains for use in subsequent agglutination tests. A suitable rabbit was then inoculated

intravenously with a small dose of the strain T.L. (day 1), and on the eighth day was inoculated with an equal dose of the strain T.T. The results of agglutination tests of its serum are presented in Chart V.

It will at once be seen that as the result of these tests the four strains of *B. typhosus* chosen at random divide themselves at once into two groups, T.E. and T.L. forming one group, and T.O. and T.T. the other. The groups are widely differentiated by the agglutination tests made during the period of primary immunisation. They remain equally



Charts II, III, IV. Agglutination Titre of Serum in three Rabbits under successive immunisation with three strains of *B. typhosus* in different orders of succession.

differentiated after the secondary antigen has been introduced. The agglutination titre rises to about 1 in 8000. And it will be seen that if in view of this fact one decided to ignore agglutination below 1 in 1000 (about one-eighth of the maximum titre) or to put it aside as "group-agglutination," one might claim that T.E. and T.L. were specifically different types from T.O. and T.T.

But if, as is to be presumed, that claim would absolutely fail to gain

support among bacteriologists, it seems not unreasonable to refuse to accept similar evidence as adequate in the case of meningococcus. In the latter case great weight appeared to be attached to the fact that the titre for Type I, the primary antigen, continued to fall when the secondary antigen was introduced, and the curve of its agglutination showed a progressive rise. Precisely the same observation holds for

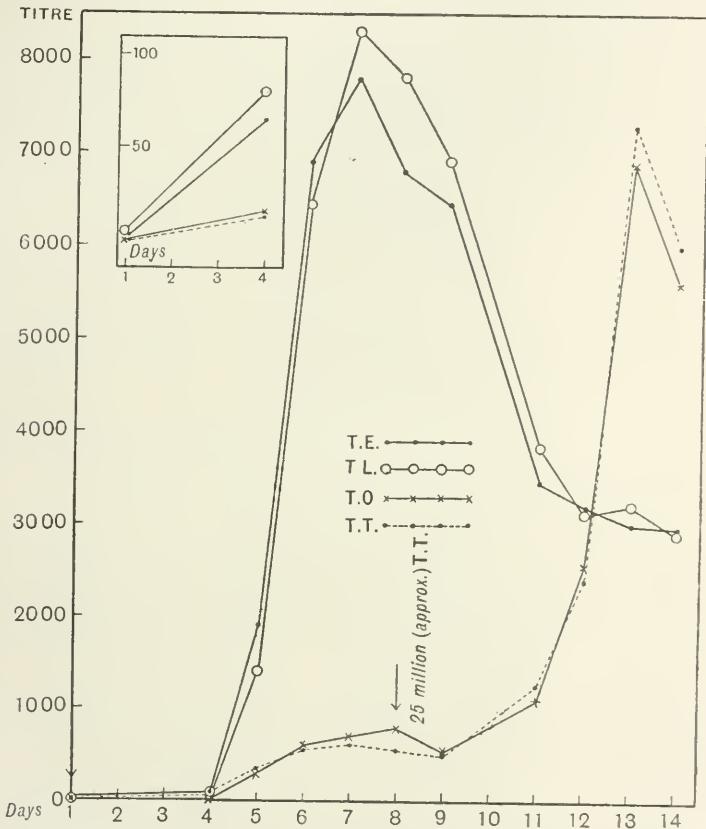


Chart V. Agglutination Titre of Serum of Rabbit tested against four strains of *B. typhosus* during successive immunisation with two of the strains.

the titre of T.L. (and its ally T.E.) when the titre for T.T. (and its ally T.O.) is rising.

It is not any part of my present intention to discuss the meaning of the very striking differences in agglutinability exhibited by these four strains of *B. typhosus* under the experimental conditions just described. They are associated with other interesting and well-marked characteristics, which do not bear immediately upon the question at

issue. But it would seem obvious that the results recorded above go a long way to deprive similar observations on the meningococcal types of conclusive value in support of the theory that the four types constitute four independent bacterial species.

ABSORPTION TESTS.

The Absorption test is applied by Lieut.-Colonel Gordon as the final and decisive means of establishing the specificity of the four types of meningococcus, and as the method to be employed for properly placing strains which are found to agglutinate with two or more type serums. For this purpose the test has been extensively made use of by Captain Tulloch who carried out an important series of laborious tests on 356 strains of meningococcus, almost all of which he succeeded in placing by this method.

How great is the value attributed to this test as a criterion of differentiation is shown by the fact that Lieut.-Colonel Gordon dismisses the opinion of M. Nicolle of the Pasteur Institute that Types I and III belong to the meningococcus group, and Types II and IV to the group of parameningococcus solely on the ground that no absorption tests were performed. On the other hand the results of absorption tests recorded by the expert workers for the Local Government Board are somewhat hastily put aside chiefly on the grounds, as it would seem, that the test is a difficult one to perform accurately, and that certain pitfalls may have been overlooked by these observers. It is, however, impossible to ignore so great an accumulation of experimental data, especially since the results of the several observers show a substantial agreement. These results are the more important because they include the investigation of certain phenomena which Lieut.-Colonel Gordon either disregards or dismisses briefly as "irregularities."

Much, of course, depends upon what is meant by an "irregularity." If it implies an error of technique it will disappear on improving the technique and repeating the observation several times. But if it means that the result obtained came out contrary to expectation, it may be that the expectation was fallacious. Such irregularities, if numerous enough, may necessitate a modification of our working hypothesis, or may invalidate a whole theory.

If the four types of meningococcus are to be regarded as "independent entities" one of the most striking irregularities is that recorded by Lieut.-Colonel Gordon himself, in which a particular strain of meningococcus absorbed the agglutinins of two separate types. It was apparently two independent entities at once.

My own experiments have revealed a number of irregularities of interest. Whether they represented reality, or were due to technical error, must remain for the time being a matter of opinion.

But for what it is worth one of the most highly paradoxical (on the view that the types are fixed and independent) is recorded in Table IX.

Two portions of the serum of a rabbit immunised against Type II and Type III were diluted to 1 in 20, the one with normal saline solution, the other with a formalised emulsion of Type II (Franklin) meningococcus of three times the opacity of the Central C.S.F. Laboratory emulsions used in the subsequent agglutination tests. The tubes were properly protected, and placed in a water-bath at 55° C. for 24 hours, and then in the cold chamber for another 24 hours. Both tubes were then centrifugalised to complete the deposition of the cocci in the second sample. Parallel agglutination tests were then made.

TABLE IX.

*Immune Serum for Type II and Type III absorbed with Type II.
Agglutination Test.*

Coccus		Serum 1 in 40	80	100	200	400	800	2000	Control
Type I	before absorption	<i>p</i> -	<i>tr</i> +	<i>tr</i>	0	0	0	0	0
Batch F	after	„ <i>p</i> -	? <i>tr</i>	0	0	0	0	0	...
Type II	before	„ <i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i> -	<i>p</i> -	0	0
Batch E	after	„ <i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i> -	<i>tr</i>	0	...
Type III	before	„ <i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>p</i>	<i>tr</i>	0	0
Batch D	after	„ <i>p</i> -	? <i>tr</i>	0	0	0	0	0	...
Type IV	before	„ <i>t</i>	<i>p</i> -	<i>tr</i> +	? <i>tr</i>	0	0	0	0
Batch C	after	„ <i>tr</i>	0	0	0	0	0	0	...

The titre of the serum for all four types of meningococcus was somewhat reduced by absorption with Type II. But the reduction was only slight¹ in the case of Type II itself, and in that of Type I. It was considerably greater in the case of Type IV, and in that of Type III, it was great enough to remove all traces of agglutination above 1 in 80. In fact were significance attached only to "complete" agglutination, one could be entitled to say that absorption with Type II coccus had left the agglutinins for Type II unaltered, while entirely removing those for Type III and Type IV. It may be added that the readings found for the non-absorbed sample of serum were substantially the same as those originally given by the same serum a fortnight earlier.

In regard to the foregoing experiments the only possible alternatives

¹ Where agglutination tests are not carried to an end-point such degrees of absorption will often be missed.

in explanation are either that the whole result was due to error of technique, or that the absorbing coccus Type II had ceased to be specifically Type II, and reacted in the manner expected of Type III. The former explanation might be accepted if the experiment stood alone. But in view of all the evidence presented above against the theory of specificity of the types, it seems quite possible that the results were reliable.

CONCLUSIONS.

1. Fixity of type among meningococci of Types I, II, III and IV (Gordon) is non-proven. Exceptions are on record and evidence is accumulating in a contrary sense.

2. Results obtained by the method of "saturation" are rendered dubious by the fact that death may be due to mechanical complications such as widespread thrombosis in the circulation. But so far as it goes it tends to show that the protection afforded by immunisation with meningococci of any type is multivalent rather than univalent.

3. The method of "superimposition" tests gives results with the four types of meningococcus, which can be paralleled with four strains of *B. typhosus*. It, therefore, affords no support to the theory of specificity of type in meningococci.

4. The serological results obtained with type meningococci in different animals (horse, man) may entirely fail to accord with types employed as antigens. Accordingly the types differentiated in relation to the rabbit do not appear to represent independent entities.

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ON THE CAUSE OF DEATH AS DETERMINED BY MICROSCOPICAL EXAMINATION IN THREE ANIMALS KILLED IN THE COURSE OF "SATURATION" TESTS.

BY A. G. GIBSON, D.M. (OXON), F.R.C.P., MAJOR R.A.M.C.T.

A Report to the Medical Research Committee.

IN a paper by Capt. Ainley Walker in this *Journal*¹ reference is made to three experiments on three rabbits killed by the "saturation" method of Lieut.-Colonel Gordon which consists in injecting into the auricular vein slope cultures of organisms in massive doses at frequent intervals. The organisms used in the present experiments were the *Meningococcus*, *B. typhosus*, and *Sarcina lutea*. Capt. Ainley Walker has handed over to me the organs of the rabbits used for histological examination as to any evidence that might exist of the cause of death. The organs had all been fixed in alcohol. Portions of each were cut out and embedded in the usual way in paraffin. The sections made were stained by haematoxylin and eosin and by such other methods as the appearances suggested. It will be best to set out in detail the features of each organ and then to summarise the whole at the end.

(1) RABBIT KILLED BY "SATURATION" WITH MENINGOCOCCUS CULTURE.

Lung. In all sections there are several patches of haemorrhage. The arteries are either empty or contain small portions of massed red cells and there are a larger number of leucocytes than normal. The intima shows swelling and proliferation of cells. The periarterial lymphatics are distended with blood. The bronchi show slight epithelial catarrh. The veins contain much clot and some of the smaller ones are turgid with clot that contains many leucocytes. The capillaries everywhere contain leucocytes and endothelial cells but very few red cells are seen. In many can be seen plugs of fibrin which is very clearly seen by the low power in any section of the lung stained by Weigert's fibrin method, the deep blue dots and tags of fibrin standing out against the

¹ E. W. Ainley Walker, *Journal of Hygiene*, Cambridge, 1918, Vol. xvii, p. 389.

red of the carmine used as a counterstain. Some of these plugs can be found partially extruded into a vein.

Kidney. Normal in general. Round cells are numerous in the capillaries and glomeruli where an occasional embolus is seen. Catarrh is present in the arterial intima. Weigert's stain shows glomeruli that contain fibrinous clot in some of their capillaries.

Liver. Early fatty infiltration. Round cells are present in capillaries. A few rounded emboli are seen in the capillaries and numerous filaments of fibrin stretching in their lumina and in some instances completely filling them.

Suprarenals. Some fibrinous emboli and fibrinous strands in the capillaries.

(2) RABBIT KILLED BY "SATURATION" WITH *B. TYPHOSUS*.

Lung. The description for meningococcus is the same as that for Typhoid except for a diminished intensity of the process. Gram negative bacilli often as diplo-bacilli can be seen in the capillaries and in the veins of the neighbourhood of the leucocytes.

Suprarenal. Some capsular haemorrhage otherwise nothing abnormal.

Liver. A good deal of fibrin of loose texture in capillaries. Under an oil immersion single threads can be seen lying on many capillaries. A few obvious fibrinous emboli, of looser texture than in meningococcal liver.

Kidney. A very few small fibrinous emboli in the capillaries of the glomeruli and a few strands of fibrin also in some of the veins, otherwise no great abnormality.

Heart. The coagulated blood (red clot) in the left ventricle shows numerous fibrinous emboli around one or more leucocytes. No emboli seen in heart substance.

(3) RABBIT KILLED BY "SATURATION" WITH *SARCINA LUTEA*.

Lung. Most of the larger veins show a mass of clot slightly shrunken away from the walls, white mixed, and red, with large numbers of polymorphs in the white parts. These show small collections of cells which presumably have been disgorged from the capillaries. The arteries are empty for the most part but contain portions of fibrinous clot; but the most marked feature is the enormous dilatation of the peri-arterial lymphatics which contain fibrinous red clot. Many arteries show marked acute intimal swelling.

There is a marked collection of polymorphs in the capillaries which for the most part contain but few red cells, in fact they are choked with polymorphs and plugs of fibrin which can be well seen in a fibrin stained preparation. Shaggy small fibrinous clots are contained in the alveoli which are otherwise empty, but a few are filled with blood. Numerous capillary emboli are seen in the Weigert stained sections. Organisms are seen embedded in fibrinous emboli though some care is necessary in detecting them.

Kidney. Most of the veins show recent fibrinous, mixed or red clot. The fibrinous clot shows a high proportion of leucocytes, and in the recent red clot are to be seen portions of fibrinous clot which have obviously been formed at some earlier period. The clot does not completely fill the lumen, suggesting a retraction from the original bulk. The larger arteries are either empty or contain some fibrinous clot. The glomeruli are diminished in size and show a marked space between glomeruli and Bowman's capsule, their capillaries as shown by Weigert's method contain fibrin and some red cells but are only poorly filled. The convoluted tubules show slight catarrh. In the general capillaries there is hardly any blood. Some glomeruli are hardly affected, in others the capillaries are nearly all plugged with fibrinous emboli.

Liver. The hepatic and portal veins are filled with clot, white, red or mixed, the fibrinous portions of the latter showing many leucocytes. Many of these portions are obviously emboli from fibrinous clot in capillary vessels. The capillaries contain numbers of polymorphs but red cells have to be carefully sought for. A Weigert stained preparation shows numerous fibrinous embolic plugs, tags and strands in the capillaries.

Suprarenals. Haemorrhage showing thrombosis just exterior to the capsule. The capillaries contain some fibrin and sarcinae but very few normal red cells.

The veins of the medulla contain white or red clot.

Brain. Nil.

REMARKS.

It will be evident from a perusal of the foregoing details that all three animals present the same features. In the lung there is a widespread capillary embolism which has led to haemorrhage varying in amount, being most evident in the rabbit killed with meningococcus. The preparations made for the express purpose of staining fibrin show these emboli most clearly even under the low power though they are

evident in the haemotoxylin stained preparation. Of these emboli a certain number become detached as may be seen in some of the pulmonary veins, they are then transmitted to the left ventricle (see typhoid rabbit) and thence may be carried to the various organs, *e.g.* kidney, liver, suprarenal. The fibrinous emboli are in many cases associated with cells and it is legitimate to suppose that by the injection of such massive doses of bacteria and their endo-toxins into the venous system some inflammation in this and in the branches of the pulmonary artery is produced sufficient to induce thrombosis.

All the animals underwent the same treatment, died with the same symptoms and their organs show an identical morbid anatomy. Two views may be put forward as to the cause of death: first that it is due to multiple capillary pulmonary embolism, and secondly that it is due to the toxic effects of the substances injected. When we consider however that one organism (*Sarcina lutea*) is not usually pathogenic at all, and that none are normally pathogenic to the rabbit, one is forced to the conclusion that the cause of death is the cause which can be readily demonstrated by the microscope, namely multiple capillary embolism of the lungs and secondarily of other organs.

A "REVERSION" PHENOMENON IN BACTERIAL FERMENTATION.

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THE publication of the following brief note is necessitated by the circumstance that, in a recent paper, Cunningham and King (1917)¹ make this remark in the course of a discussion of the question of acquisition of new fermentative properties by bacteria: "It would appear however that the characteristic once acquired is not absolutely permanent, for Ledingham has shown that the reverse process can take place, alkaline papillae appearing on colonies which have been thus trained." In a footnote the authors add, "We have been unable to find the reference to this piece of work. Col. Ledingham told one of us (J. C.) about it in the course of a conversation." This observation referred to has not hitherto been published as it emerged simply in the course of a larger investigation which was interrupted by the war. In their statement, Cunningham and King do not perhaps quite strictly express the nature of the reversion process as it was observed by me in a strain of *B. dysenteriae*, and consequently I give the relevant facts here while postponing their full discussion to a more convenient season.

SOURCE OF MATERIAL INVESTIGATED.

Two strains of *B. dysenteriae* (mannite-fermenting type) which had been isolated from cases of asylum dysentery by D. McKinley Reid (1913)² were given me for further study.

Both strains were agglutinated readily by stock Flexner-Y serum but a specific serum prepared by immunisation with one strain "A" had only feeble action on strain "B" and *vice versa*. The two strains differed also with respect to their fermentation reactions on certain carbohydrate media, the chief differences being in connexion with

¹ Dysentery in the jails of Eastern Bengal, *Indian Journ. Med. Res.* v. 103.

² On the Bacteriology of Asylum Dysentery in England, *Journ. of Ment. Sc.* LIX. 621.

raffinose, arabinose, and isodulcite. From extracts of my notes available here (Baghdad) I give the following particulars of these differences.

FERMENTATION PROPERTIES OF ORIGINAL STRAINS.

Raffinose:

Strain "A." Examination of 10 separate colonies inoculated simultaneously on tubes of raffinose peptone water. One gave full acidity in three days, one in four days, four in six days, one in seven days, one in eight days and two in twelve days. The limits of time, therefore, for development of full acidity, were three to twelve days.

Strain "B." Examination of 10 colonies as before. Seven gave full acid reaction in two days and the remaining three in four days.

Isodulcite:

Strain "A." Examination of 5 colonies. All gave full acidity in 24 to 48 hours.

Strain "B." Examination of 5 colonies. One showed full acidity on the fourteenth day while the remainder showed no change during a period of observation of 20 days. From the variant which produced a full acid reaction on the fourteenth day, was ultimately obtained one which gave full acidity on isodulcite in five days. When plated out on solid medium containing isodulcite, certain of the white colonies after prolonged incubation (nine days) developed red papillae—a phenomenon now well known and frequently studied. It will not be referred to further here.

Arabinose:

Strain "A." All colonies gave full acidity in 24 hours.

Strain "B." The ten colonies examined showed no change whatever at least in fourteen days but it should be added that an acid-forming variant was ultimately derived from this strain.

The differences noted above are grouped together for convenience in the table annexed.

Raffinose		Isodulcite	Arabinose
"A"	Full acid in 3-12 days	Full acid in 24-48 hours	Full acid in 24 hours
"B"	Full acid in 2-4 days	Acid-forming variant selected by prolonged growth on medium (14 days). Development of red papillae on white colonies of ditto	No change in 14 days. Acid-forming variant selected by prolonged growth on medium

With these differences before us, the question arose whether variants could be obtained from the original strains "A" and "B," which would show a similar behaviour on the three substances employed and, if so, whether such approximation was accompanied by a serological *rapprochement* in cross-agglutination experiment. From a consideration of the original properties of the strains, it will be realised that the

selection of a variant promptly fermenting raffinose, was readily evolved from "A" while from "B" it was at least possible to secure a variant rapidly attacking isodulcite and arabinose, by the simple expedient of working from acid papillae developing on white colonies after long or short periods of incubation. The method, however, was applied only in the case of "B" on arabinose, when a variant producing early acidity on this substance was ultimately obtained.

The peculiar behaviour of "A" on isodulcite plates afforded a much simpler method of securing identical variants so far as this medium was concerned. Strain "A" was inoculated on isodulcite peptone water, and at various periods of its growth the culture was pla'ed out on isodulcite agar containing bile salt.

RESULTS, WITH DESCRIPTION OF "REVERSION" PHENOMENON.

3 days' growth (full acid reaction). Plates yielded mainly white colonies on the first day of incubation but a few had an intense red colour. On the second day all the colonies were intense red. On the third day white papillae were appearing on the surface of certain of the red colonies. The papillae attained fairly large dimensions as incubation proceeded.

5 days' growth. Plates sown from the culture at this date, yielded mainly white colonies after 24 hours' incubation, with however a few intense red colonies. On the second day all the colonies had an intense red colour. On the third day white papillae appeared on certain of the red colonies.

7 days' growth. Plates at the end of 24 hours' incubation showed a mixture of red and white colonies. On the third day, all the colonies were red and papillae had appeared on some of these. It was observed that those colonies which developed an intense red colour in 24 hours, yielded no papillae.

10 days' growth. Plates yielded after 24 hours' incubation almost solely red colonies with only a few whites. After 48 hours, all the colonies were red. On the ninth day a minority of the red colonies had developed papillae, the proportion being 5 papillated to 30 non-papillated.

13 days' growth. Plating of one loop of the culture gave only two colonies after 24 hours, the one intense red and the other white. After 48 hours both were red. On the fourth day the colony which last became red was studded with white papillae while the original red colony showed none.

14 days' growth. Plating of three large loops of culture yielded on the first day reds and whites in the proportion of 13 to 54. On the third day all were red, but only those colonies which last became red had developed white papillae. The early intense reds showed none.

17 days' growth. Plating of 1 c.c. of the culture gave no apparent growth after 24 hours but on the second day many colonies of the less intense red type appeared. The majority of the colonies were, however, still white. On the fourth day all the colonies had developed a red colour and papillae had appeared on most of them.

Plating of the isodulcite fluid culture after this date yielded no growth. The whole contents of the tube were inoculated into broth which remained sterile. The isodulcite fluid culture thus lived 17 days but not 20 days.

We thus see that strain "A," which, on analysis of several colonies had been shown to give a full acid reaction on fluid isodulcite medium in 24 to 48 hours, exhibited parallel variations on plates, some of the colonies showing an intense red colour in 24 hours while the others developed the red colour only at the end of 48 hours' incubation. Full acidity on the plates was rarely longer delayed. The strain would ordinarily be regarded as a fairly prompt fermenter of isodulcite. Plating however showed that the strain could be split up into two distinct elements, viz. (1) producing acid very rapidly from isodulcite and (2) producing somewhat less acid and only after 48 hours' incubation. The colonies of (2) also had the property of developing secondary white colonies on the surface, sometimes in enormous numbers, whereas the early acid-formers invariably produced none. On the tenth day of growth on fluid isodulcite, plating showed a marked preponderance of type (1) or the non-papillae-producing type, but after this date, plating of the isodulcite fluid showed a decline of the intense acid-formers and a relative preponderance of the papillae-producing types.

These were the last viable survivors on the seventeenth day.

ANALYSIS OF THE TWO TYPES.

The colonies taken for examination were those obtained by plating of the isodulcite fluid on its thirteenth day of growth (see above), viz. (1) intense red non-papillated colony, (2) red colony with white papillae.

Exp. 1. The red central portion of (1) was touched with a needle, inoculated on ordinary broth and finally plated on isodulcite bile salt agar. Result: On the first day all the colonies were intense red. On the second day the colonies were becoming alkaline. No further change occurred except that the colonies showed colour alterations pointing to alkaline reaction. All the colonies remained smooth.

Exp. 2. A portion of the white periphery of the same colony (1) was touched and plated. Colonies exactly similar to those of *Exp. 1* resulted.

Exp. 3. A white papilla from (2) yielded white colonies solely, some of which on the sixth day showed extremely minute secondary white papillae on their surface.

Exp. 4. The red portion of (2) yielded on the first day, white colonies only. On the second day many had turned red and were showing white papillae. On the sixth day there still remained a number of pure white colonies in addition to the red types with white papillae.

FURTHER TESTING OF COLONIES FROM EXP. 3.

Four white colonies from this experiment were inoculated on isodulcite peptone water. For 17 days no trace of acid reaction occurred in any. Between the seventeenth and thirty-first days two of the tubes began to show an acid reaction but, on the thirty-first day (the last day of observation) the medium in two of the tubes remained unaltered.

We have therefore demonstrated the presence of three different elements in strain "A" with respect to action on isodulcite, viz. (1) an element fermenting the substance rapidly (in 24 hours) with intense acid production and subsequently rendering the medium alkaline; (2) an element fermenting the substance less rapidly (48 hours) and throwing off subvariants (3) which have no action whatever on isodulcite and are detected by their occurrence as white papillae on the surface of their red ancestors.

The two elements (1) and (2) were obtained in a pure state and the non-acid-producing subvariants (3) remained stable for the period of 31 days during which the observations were continued.

I do not propose to discuss this interesting type of variation further at this stage as I have access here only to extracts from my original notes, but I may add that the two final variants selected from the original strains "A" and "B" and used for immunisation of rabbits gave the following reactions on the three substances raffinose, isodulcite and arabinose. Period of observation on fluid media, 21 days.

	Raffinose	Isodulcite	Arabinose
Variant from "A"	A (24 hours)	—	A (48 hours)
Variant from "B"	A (48 hours)	—	A (48 hours)

This was the closest approximation obtained when the experiments were interrupted. A more complete approximation was prevented by the development on the part of "A" of a tendency to throw off lactose variants and these lactose elements could not so far be eliminated from the final "A" variant used for immunisation.

Sera prepared from the final variants gave the following reactions in cross-agglutination experiment. The reactions with the sera prepared by immunisation with the original strains, are also adduced.

End-point readings after 24 hours

Serum from "A" (original) v. "A" (original)	1 in 6400
Serum from "B" (original) v. "B" (original)	1 in 6400
Serum from "A" (original) v. "B" (original)	1 in 200
Serum from "B" (original) v. "A" (original)	1 in 400
Serum from A' (final variant) v. A'	1 in 12800
do. do. do. v. B'	1 in 400
Serum from B' (final variant) v. B'	1 in 25600
do. do. do. v. A'	1 in 3200
Serum from "A" (original) v. A'	1 in 3200
do. do. v. B'	1 in 100
Serum from "B" (original) v. B'	1 in 3200
do. do. v. A'	1 in 200

Thus, the sera prepared from the variants, though of higher titre than those prepared from the originals, behaved in a similar manner with respect to the variants, and absorption experiments not here detailed revealed little or no absorption of the specific agglutinins of the variant by digestion of its homologous serum with the heterologous final variant.

SUMMARY AND CONCLUSION.

Two strains of *B. dysenteriae* isolated from cases of asylum dysentery, were found to be readily agglutinable with stock Flexner-Y serum but serum prepared from the one had only a feeble action on the other. Absorption experiments revealed a like specificity. They differed in certain particulars in their action on certain carbohydrate media, and the attempt was made to obtain, by various procedures, variants from each strain giving fermentation reactions as alike as possible.

In the course of this work, an interesting and probably hitherto undescribed type of variation was met with, viz. the capacity exhibited by one strain which fairly promptly fermented the substance employed (isodulcitol), of throwing off variants having no action on the substance. These variants appeared as white papillae on the surface of the original red ancestor and the description and analysis of this "reversion" process form the main interest of this paper. Though a fairly close approximation in fermentation characters was realised by selection of variants from each of the original strains, little or no approximation was apparent in respect of the serological affinities of these final variants.

BAGHDAD,
January, 1918.

THE ABSORPTION OR SATURATION TEST OF CASTELLANI: ITS APPLICATIONS IN SERO-DIAGNOSIS, AND IN THE RECOGNITION OF BACTERIAL SPECIES.

A CRITICAL REVIEW.

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THE Absorption or Saturation Test published by Castellani in 1902 is now becoming more extensively used by serological and bacteriological workers in European and American laboratories. Its use is also essential for the accurate study of certain tropical infections, so that it behoves all tropical workers to be familiar with this test. It is therefore believed that an account of the method together with a general review of the literature of the subject may not be out of place in this *Journal*.

The phenomenon of absorption of agglutinins from serum on saturation with homologous organisms was first observed by Bordet (1899) in the case of normal serum. Bordet's observation was soon confirmed by Eisenberg and Volk (1902).

Castellani (1902) next discovered that the serum of rabbits immunised with *B. typhosus* agglutinating not only the typhoid bacillus, but also to a less extent certain strains of *B. coli*, on saturation with an excess of typhoid bacilli not only lost its specific (primary homologous) typhoid agglutinin, but also the non-specific (secondary heterologous or co-agglutinin) coli agglutinin. Saturation of the same serum with *B. coli* was found to remove the non-specific coli agglutinin, but the specific typhoid agglutinin was not removed, or only to a very small extent. Further, in a serum derived from rabbits immunised both for *B. typhosus* and *B. coli*, saturation either with typhoid bacilli alone or with *B. coli* alone failed to remove the whole of the specific agglutinins, whilst this was achieved by saturation with both organisms either simultaneously or successively.

The following experiments selected from those recorded by Castellani will illustrate these findings—a rabbit immunised to *B. typhosus* agglutinated *B. typhosus* 1:5000 and *B. coli* (31) 1:600. After saturation

with typhoid bacilli, all agglutinins were removed for both micro-organisms. A rabbit immunised to both *B. typhosus* and *B. coli* (31) agglutinated typhoid bacilli 1:4000 and *B. coli* (31) 1:1000. After saturation with typhoid bacilli the serum no longer agglutinated typhoid, but did agglutinate *B. coli* (31) 1:900. After saturation with the colon bacillus (31) it failed to agglutinate *B. coli* (31), but still agglutinated *B. typhosus* 1:4000.

From these and other experiments Castellani drew the following important conclusions:—(1) The serum of an animal immunised against a certain micro-organism, when saturated with that micro-organism, loses not only its agglutinating power for that organism, but also for all the other varieties that it acted upon; when saturated with the others, its agglutinating power upon the first is reduced little or not at all. (2) The serum of an animal immunised against two micro-organisms, *A* and *B*, loses its agglutination when saturated with *A* only for *A*. Saturated with *A* and *B* it loses its agglutinating power for both. (3) These facts may be applied to the diagnosis of mixed infections, and to the differentiation of closely allied germs. Suppose for instance the serum from a typhoid case agglutinates with the laboratory culture of *B. typhosus* and those of a variety of *B. coli*, saturate the serum with typhoid bacilli. If the serum loses its agglutinating power for the typhoid bacillus only, it is a case of mixed infection with both the typhoid and colon bacilli. If the serum loses its agglutination for both the typhoid and coli organisms, it is a pure typhoid infection, the *B. coli* having been agglutinated by the group agglutinins produced by the typhoid infection.

These findings of Castellani were not immediately accepted without criticism. Thus, Posselt and Sagasser (1903) showed that in immunising there is not only an increase in the amount of primary agglutinins for the organism used but also of secondary agglutinins which act on other organisms. As regards absorption these secondary agglutinins behave like the special agglutinins in cases of mixed infection and as the agglutinins in normal serum, being often increased to a high degree. Thus the serum of a guinea-pig immunised against *B. typhosus* with a titre of 1:12,000 for this bacillus had also secondary agglutinins for *B. cholerae* 1:4500 and for *B. dysenteriae* 1:4000.

Ballner and Sagasser (1904) also showed that a homologous bacterial species can withdraw from an immune serum only its own primary agglutinins but not the secondary agglutinins which act on other bacteria, and that a heterologous bacterial species binds only its own partial

agglutinins and no other portion of the total agglutinins; hence they conclude that the absorption of agglutinins through homologous and heterologous micro-organisms must be regarded as a strong specific reaction. They give examples showing that the co-agglutinins are at times markedly increased, and that inoculation with *B. tetani* and *B. pneumoniae* of Friedländer lead to the formation of few primary agglutinins, but numerous co-agglutinins.

Again, Hetsch and Lentz (1903) by employing the method of absorption to genuine cholera bacilli and cholera-like vibrios, demonstrated the specificity of the agglutinins in normal horse serum and in that of an animal immunised against the *B. cholerae*. Saturation with *B. cholerae* diminished the agglutinins for this organism whilst the co-agglutinins remained either the same or were only slightly diminished.

Levy and Fornet (1908) also insisted on the value of Castellani's saturation method of distinguishing primary and secondary agglutinins, both in patients' and in animal sera, provided always that high serum dilutions were repeatedly treated with large amounts of bacteria.

D'Amato (1910) also, as the result of a very extensive investigation, came to the conclusion that, by means of the saturation test it is possible to determine with considerable certainty which is the real infecting organism in doubtful cases of typhoid infection.

The importance of co-agglutinins and of their investigation in cases of mixed infection was also recognised by Dreyer (1916) who made use of standard agglutinable cultures in testing the agglutinating power of serum against *B. dysenteriae* (Shiga, Flexner and Y), *B. typhosus*, *B. paratyphosus* A, *B. paratyphosus* B, *B. enteritidis* (Gaertner), *B. coli* and *Vib. cholerae*.

When in the course of a differential diagnosis it is found that the patient shows a high agglutination titre for two or more of the dysenteric bacilli, the question arises whether we are dealing with co-agglutination, or with agglutination persisting from a past infection with another form of bacillary dysentery or with a mixed infection. In the former case the rise and fall in agglutination to the different microbes will be synchronous. Dreyer considers that it is more common to see a co-agglutination of *B. Flexner* in a *B. Shiga* infection than of *B. Shiga* in a *B. Flexner* infection. When the case is one of mixed infection the agglutination curves for the different infecting organisms are usually not synchronous, and pursue their ordinary course independently of each other.

TYPHOID-PARATYPHOID GROUP.

Conradi (1904) confirmed Castellani's findings in the case of typhoid and paratyphoid infections.

Park and Collins (1904) in a Study of "Specific and Non-Specific Agglutinins" successively applied this method to a serum mixture obtained by adding equal parts of serum from an animal injected with a maltose fermenting paradysentery culture (Manila) and from an animal injected with a paratyphoid bacillus. Their results are set forth in Table I.

This was followed by Boycott (1906) in an elaborate series of "Observations on the Bacteriology of Paratyphoid Fever and on the Reactions of Typhoid and Paratyphoid Sera."

The accuracy and value of the Castellani test he stated to be generally acknowledged and he considers that absorption tests are necessary for the positive or negative diagnosis of mixed infections. Some of his results are thus set forth (Table II).

These figures, and others given, he considers require little comment.

Bainbridge (1909) published a paper "On the Paratyphoid and Food-poisoning Bacilli, and the Nature and Efficiency of Certain Rat Viruses," in which he employed this method extensively "in the hope of obtaining more complete differentiation of these bacilli than is afforded by their agglutination reactions."

Some of his results are exhibited in Table III.

Harvey (1909) investigated the enteric fever convalescents at the dépôt at Naina Tal and found that a certain number of these were *B. paratyphosus* A infections. In one case where *B. paratyphosus* A was isolated from the blood and faeces he performed absorption experiments (Table IV) in order to differentiate the specific and group agglutinins.

It will be noted that the high agglutinins for *B. typhosus* were almost removed by absorption with *B. paratyphosus* A isolated from the patient's own blood. If these agglutinins had been due to the *B. typhosus* then absorption with *B. paratyphosus* A should have only slightly reduced the agglutination titre. Absorption with the *B. typhosus* reduced but did not remove the low agglutinins for the *B. paratyphosus* A.

In another case which was proved by culture to be a double infection with the *B. typhosus* and the *B. paratyphosus* A, absorption with the latter bacillus had no effect on the agglutinins for the former.

Notwithstanding these results Harvey concludes that the only certain method by which cases of infection by paratyphoid bacilli can be differentiated from cases due to infection by the *B. typhosus* is by careful

TABLE I.
Absorption by the paratyphoid bacillus.

	Agglutination	
	Before Absorption	After Absorption
Paratyphoid bacillus	1 : 500	< 1 : 10
Dysentery bacillus	1 : 1000	1 : 800
Colon bacillus X	1 : 500	1 : 10

TABLE II.

Nature of serum	Dilution	Absorbed with	Agglutination (after absorption) with					
			<i>Typhoid Guy's</i>	<i>Brion and Kayser</i>	<i>Schott B.</i>	<i>Aertryke</i>	<i>Gaertner < I.P.M.</i>	<i>Schott A.</i>
I. Typhoid: human	1 : 10	Original titre	2000	1000	50	50	500	50
		<i>Typhoid Guy's</i>	{ +++ ○	{ ○ ○	{ ○ ○	{ ○ ○	{ +++ ○	{ - -
		<i>Brion and Kayser</i>	{ +++ +++	{ ++ ○	{ ○ ○	{ ○ ○	{ +++ +++	{ - -
		<i>Schott B.</i>	{ +++ +++	{ ++ ○	{ ○ ○	{ ○ ○	{ ++ ++	{ - -
		<i>B. coli communis</i>	+++	++	+++	++	+++	-
II. Para- typhoid B: human case "Barklay"	1 : 10	Original titre	200	500	75,000	1000	200	50
		<i>Typhoid Guy's</i>	○	+++	+++	+++	+	-
		<i>Brion and Kayser</i>	{ ++ ++	{ +++ ○	{ +++ +++	{ +++ +++	{ +++ +++	{ - -
		<i>Schott B.</i>	{ ○ ○ ○	{ ○ ○ ○	{ +++ +++ ○	{ +++ +++ ○	{ ○ ○ ○	{ - - -
		<i>Aertryke</i>	○	○	+++	○	○	-
		<i>Gaertner < I.P.M.</i>	○	+++	+++	+++	○	-
		<i>B. coli communis</i>	+++	+++	+++	+++	+++	-
		<i>Guy's and Brion and Kayser + Aertryke + Gaertner</i>	{ ○ ○	{ ○ ○	{ +++ ○	{ ○ ○	{ ○ ○	{ - -
III. Gaertner: original rabbit	1 : 25	Original titre	1:1000	200	20	<20	5000	50
		<i>Typhoid Guy's</i>	○	○	○	-	+++	-
		<i>Brion and Kayser</i>	++	○	○	-	+++	-
		<i>Schott B.</i>	+++	○	○	-	+++	-
		<i>Gaertner < I.P.M.</i>	○	○	○	-	○	-
		<i>Aertryke</i>	+++	○	○	-	+++	-
		<i>B. coli</i>	+++	+	○	-	+++	-
IV. Aertryke: rabbit	1 : 50	Original titre	200	500	2000	2000	20	-
		<i>Typhoid Guy's</i>	○	+++	+++	+++	-	-
		<i>Brion and Kayser</i>	○	○	+++	+++	-	-
		<i>Schott B.</i>	○	+	○	+++	-	-
		<i>Aertryke</i>	○	○	○	○	-	-

TABLE III.

Serum		Agglutination at 1-200 after absorption		
		<i>B. Aertryke</i>	<i>B. Paratyphoid B</i>	<i>B. Suipestifer</i>
<i>B. Aertryke</i>	Original titre	5000	5000	2000
	Absorbed with:			
	<i>B. Aertryke</i>	○	○	○
	<i>B. Suipestifer</i>	○	○	○
	<i>B. Paratyphoid B</i> { 1 : 2000	+	○	+
	{ 1 : 1000	+	○	+
<i>B. Paratyphoid B</i>	Original titre	5000	1000	1000
	Absorbed with:			
	<i>B. Paratyphoid B</i>	○	○	○
	<i>B. Aertryke</i>	○	+	○
	<i>B. Suipestifer</i>	○	+	○
<i>B. Suipestifer</i>	Original titre	5000	4000	5000
	Absorbed with:			
	<i>B. Suipestifer</i>	○	○	○
	<i>B. Aertryke</i>	○	○	○
	<i>B. Paratyphoid B</i> { 1 : 200	+	○	+
	{ 1 : 2000	+	○	+
<i>B. Aertryke</i>	Original titre	4000	4000	4000
	Absorbed with:			
	<i>B. Aertryke</i>	○	○	○
	<i>B. Suipestifer</i>	○	○	○
	<i>B. Paratyphoid B</i> { 1 : 200	+	○	+
	{ 1 : 1000	+	○	+
<i>B. Paratyphoid B</i>	Original titre	—	10,000	10,000
	Absorbed with:			
	<i>B. Paratyphoid B</i>	—	○	○
	<i>B. Suipestifer</i>	—	+	○

TABLE IV.

Pte *P*'s serum untreated.

Widal reaction	20	40	100	200	400
Stock typhoid	+	+	+	±	∓
Para A own strain	±	∓	Trace	—	—

Pte *P*'s serum after absorption for one hour with own bacillus:

Widal reaction	20	40	100	200	400
Stock typhoid	∓	Trace	—
Para A own strain	Trace	Trace	—

Pte *P*'s serum after absorption for one hour with the *B. typhosus*:

Widal reaction	20	40	100	200	400
Stock typhoid	Trace	—
Para A own strain	±	Trace	—

cultural examinations of the blood and excreta, and that this differentiation has a practical significance in view of the statistics for enteric inoculation as this prophylaxis cannot be expected to protect against paratyphoid infection, which would appear to be becoming more common in India.

In a later communication Harvey (1915) considered that blood culture should be attempted in every case at the earliest opportunity, but if this fails (as often happens) it is quite possible to diagnose paratyphoid fever on clinical grounds in combination with a close study of the Widal (including absorption) reactions. He also found that in cases due to infection with the *B. paratyphosus* A whose serum agglutinated both the paratyphoid A and typhoid, absorption with A removed all the agglutinins, or if the man had been inoculated reduced those for typhoid to the level that obtained before the fever. Absorption with *B. typhosus* removed only the group agglutinins for this bacillus but not those for *B. paratyphosus* A, even although these had only been present in a 1 in 20 dilution. This rule, however, is not invariable, as in some cases the titre for paratyphoid A was considerably reduced by absorption with the *B. typhosus* although the cases had been proved by blood culture to be pure paratyphoids; but this was exceptional.

The method was then assiduously employed by Officers of the Royal Army Medical Corps working in India.

Gratton and Wood (1911) investigating Paratyphoid Fever in India differentiated the typhoid from the paratyphoid bacilli by means of the absorption method. They found that if the organism tested were the *B. paratyphosus* A the specific agglutinins were completely removed. Before accepting a suspected organism as *B. paratyphosus* A they required that it should completely remove the agglutinins specific for *B. paratyphosus* A; and they frequently tested heterologous organisms as controls, such as *B. typhosus*, *B. paratyphosus* B and *B. coli* against their paratyphosus A serum, and by this means have never removed the specific agglutinins for *B. paratyphosus* A.

Gratton and Harvey (1911) investigated a small epidemic of Typhoid Fever in India caused by an "acute carrier," and identified *B. paratyphosus* A in the stools of the carrier by means of the absorption test, and state that "to accept any bacillus isolated from faeces as a paratyphoid bacillus without putting it through some such series of tests (as agglutination and absorption) is to run great risks of making mistakes."

Harvey (1911) in discussing "The Causation and Prevention of Enteric Fever in Military Service, with a Special Reference to the

Importance of the Carrier" made use of the absorption method in the identification of the typhoid and paratyphoid bacilli in the faeces of convalescents and carriers.

Firth (1911) in discussing "The Para-typhoid Problem in India" considers that "it is only by the adoption of critical methods such as Castellani's Absorption Reaction that differentiation (of the paratyphoid bacilli) is possible.

Sacquépée (1916) calls attention to the great help which may be obtained by the use of the saturation test in the diagnosis of certain cases of typhoid in which the serum contains high amounts of agglutinin.

Chantemesse and Grimberg (1916) also call attention to the great practical importance of the saturation test in certain cases of typhoid and paratyphoid fever.

Gautier and Weissenbach (1916), as the result of an elaborate study came to the conclusion that the saturation test is extremely useful in determining which is the specific agglutinin, when two or more agglutinins are present in the serum. The results obtained by the saturation test in cases of typhoid and paratyphoid infections have always been identical with the results obtained by haemoculture.

Three of the less frequent and less known members of the typhoid group discovered by Castellani, namely *B. Asiaticus* I, *B. Asiaticus* II, and *Bact. Columbensis* were experimentally differentiated from each other and from the other members of the typhoid-coli group by the use of the absorption method by Fulle (1914).

DYSENTERY.

The absorption test has also been applied to the dysentery group of bacilli and has yielded somewhat discordant results in the hands of various workers, and very different opinions have been expressed as to its value in the investigation of this group of organisms.

Thus Morgan (1911) investigated a large number of strains of the mannite-fermenting group of *B. dysenteriae* isolated in this country from sources not obviously connected with clinical dysentery. He concluded that these indigenous strains, like certain foreign strains isolated from definitely dysenteric sources, could not be completely identified with any of the well-known types of the group by the application of extensive fermentation and absorption tests. He believes that when a sufficiently extended series of carbohydrate media is tested, the fermentation properties of the mannite-fermenting group afford an indication of

differences between the members of this group which are not brought to light by agglutination and absorption tests.

Again, Park (1904) and Händel (1908) found that by the absorption method they could distinguish the Flexner and Y types from each other, and by the extensive use of homologous and heterologous immune sera showed that they each possessed, in great part, homologous agglutinins for the other types.

Somewhat similar uncertain results were also obtained by Posselt and von Sagasser (1903), by Knox and Shorer (1906) by Lüdke (1911) and by Winter (1911). The uncertain results obtained by these various investigators may be explained partly by the variable agglutinating powers usually shown by the bacillus and sometimes also by Flexner's bacillus, and partly to the fact that they consider that the method of Castellani is subject to a whole series of experimental errors due to its complicated character.

Kruse (1907), who considers the Shiga-Kruse bacillus to be the only true dysentery bacillus, and includes all the other varieties—Flexner, Strong and Hiss—under the term pseudo-dysentery bacilli, the former being spoken of as toxic and the latter as atoxic dysentery bacilli, successfully employed the absorption method in the differentiation of the atoxic or pseudo-dysentery group of bacilli. He found the Y bacillus to comprise seven types, whilst the Flexner and Strong bacilli consist only of one of each type. Nine types were thus produced, and these were designated by the capital letters A to H. In Kruse's absorption experiments, however, doubtful or anomalous results were not infrequently obtained, being ascribed by him to experimental errors.

Winter (1911) also examined thirty-one strains of the Flexner type of dysentery bacilli. Castellani's method was found unsatisfactory and cross agglutination experiments were relied upon, with the following results: Eleven strains appeared to correspond to Kruse's *B. pseudo-dysenteriae* D; seven to Kruse's group A; three were so feebly agglutinated by any of his sera, that they could not be classed with any certainty, but appeared to belong to group A; two were intermediate between groups A and D; whilst the remaining twelve could not be grouped by any of the sera employed.

Wassermann (1912), too, in employing the absorption test for the differentiation of the dysentery bacilli found similar anomalous results to occur so frequently, that he considers the method unsuitable for their differential diagnosis.

Lösener (1909) also encountered great difficulties in applying the

results of the absorption test to the differentiation of the atoxic group of dysentery bacilli. Thus, a dysentery bacillus which he isolated in Königsberg, and which gave all the cultural characters of the Y bacillus, and was agglutinated in a dilution of 1 in 5000 by a Y serum with a 1 in 10,000 titre, could not be placed by the method of absorption into any of Kruse's groups.

Lentz (1913) also isolated an atoxic Y bacillus which could not be placed by the absorption test in any of Kruse's groups and must therefore be considered as a new type, if the results of the absorption test can be taken as the criterion.

Ruffer and Willmore (1909) isolated a bacillus causing dysentery at El Tor and designated it *B. dysentericus* El Tor, No. 1. By agglutination they demonstrated the close relationship of this organism to the *B. pseudo-dysentericus* D of Kruse, and could differentiate them from each other only by saturation.

Mayer (1910) also obtained similar results.

Hutt (1913) confirmed Kruse's opinion of the futility of grouping dysentery bacilli according to their action on carbohydrates. By the use of the absorption method he also confirmed Kruse's groupings and even increased their number.

The above experimental findings would indicate that if we are to take the absorption test as our guide, the number of types of dysentery bacilli must be considerably increased—a view for which Lenz (1913) however considers that at present there is not sufficient justification. Obviously this point can only be settled by a much more extensive and systematic investigation of the subject than has yet been attempted.

CHOLERA AND ALLIED VIBRIOS.

The absorption method was applied to cholera and El Tor Vibrios by Ruffer (1907), in an attempt to decide whether the El Tor Vibrios should be considered to be true cholera vibrios or not. A thorough and complete serological examination was undertaken including the agglutination, saturation, and complement fixation tests and Pfeiffer's reaction. Undoubted cholera vibrios were positive to all these tests and were not haemolytic. The El Tor vibrios gave positive agglutination and saturation tests and Pfeiffer's reaction with cholera serum, but did not fix the cholera immune body and were strongly haemolytic. A third group of vibrios fixed the cholera immune body but were not agglutinated by immune serum and did not give the saturation test or Pfeiffer's reaction and were feebly haemolytic; whilst a fourth group of strongly

haemolytic vibrios were negative to all the serological tests. From a consideration of all the serological data Ruffer came to the conclusion that the El Tor vibrios were to be distinguished from true cholera vibrios, and considers that the agglutination, saturation, and Pfeiffer's tests are not in themselves of absolute diagnostic value for cholera vibrios.

Neufeld and Händel (1907) however after a re-examination of some of the El Tor vibrios consider that they are true cholera vibrios.

Crendiropoulo (1912) examined the stools of large numbers of passengers arriving in Alexandria from infected parts for vibrios and subjected the vibrios so obtained to thorough cultural and serological investigations, including agglutination, saturation, Pfeiffer's, and the complement-fixation tests, haemolytic power and pathogenicity to pigeons. The saturation tests gave similar results to the agglutination tests.

PNEUMOCOCCUS.

Many years ago Eyre and Washbourn (1899) showed that a given antipneumococcic serum would protect rabbits only against certain strains of pneumococci. Bezançon and Griffon (1900) confirmed this observation by experiments in both agglutination and protection.

Using the methods of Cole (1914) a similar serological study was made by Mathers (1915) of the cases of lobar pneumonia entering the Cork County Hospital during the season of 1914-15 and his results may be seen at a glance in Table V.

TABLE V.

Classification of Different Strains of Pneumococci.

Group	No. of Cases	Percentage of total	Mortality
I	50	45.0	26 %
II	25	22.5	28 %
III	5	4.5	80 %
IV	31	28.0	25.8 %

Thus Group I was the dominant and typical type, but its members are less virulent than those of Groups II and III, whilst Group IV, consisting of the heterologous types, was the least virulent of the four types.

Dochez and Avery (1915), working in New York, also found four serological types of pneumococci obtained from cases of lobar pneumonia.

Their results for 1912-13 and 1913-14 may be tabulated thus (Table VI):

TABLE VI.

	1912-13	1913-14
Group I	35 = 47 %	21 = 30 %
Group II	13 = 18 %	28 = 39 %
Group III (<i>mucosus</i>)	10 = 13 %	6 = 8 %
Group IV (heterogeneous)	16 = 22 %	16 = 23 %
Total typical	58 = 78 %	55 = 77 %
Total heterogeneous	16 = 22 %	16 = 23 %
Total	74	71

Neufeld and Händel (1910) demonstrated the existence of certain varieties of pneumococcus which were fundamentally different in their immunological reactions. Dochez and Gillespie (1913) applied the methods of protection and agglutination to the investigation of the pneumococci obtained from a series of human cases of pneumonia. In this way they were enabled to classify the pneumococci associated with lobar pneumonia in man into four groups. Groups I and II consisted of members closely related immunologically to other members of their respective groups; Group III was formed of the *Pneumococcus* or *Streptococcus mucosus*; and in Group IV the remainder were combined to form a heterogeneous group. In 35 instances they found that in 4 per cent. the organisms belonged to Group I; in 13 instances 18 per cent. belonged to Group II; in ten instances 13 per cent. to Group III and in 16 instances 22 per cent. to Group IV.

TABLE VII.

Anti-pneumococcus Serum II absorbed by	Pneumococcus										Group II Type II
	Subgroup II A				Subgroup II B			Subgroup II X			
	Jn.	As.	L.	M.	W.	Ar.	J.	S. 13	F.C.B.	H.	
Subgroup A. Jn.	-	-	-	-	++	+±	++	++	+	++	++
„ B. W.	++	++	++	+±	-	-	-	++	+	++	++
„ X. S 13	++	++	++	+±	++	++	++	-	+	++	++
Group II	-	-	-	-	-	-	-	-	-	-	-

Cole (1914) was also able to differentiate pneumococci into four groups according to their agglutination reactions and their protective power, and the methods of absorption were then applied to the pneumococci so grouped. Table VII gives the results of some of his absorption experiments with these three sub-groups of Type II which he designated sub-groups II A, II B, and II X respectively.

The organisms in these three sub-groups gave: (*a*) Agglutination with anti-pneumococcus serum II: (*b*) Protection with anti-pneumococcus serum II, except sub-group II X, and (*c*) Absorption of anti-pneumococcus serum II with a member of sub-groups II A or II B removes only the antibodies for the homologous sub-group and absorption of anti-pneumococcus serum II with any given number of sub-group II X removes the antibodies for that particular strain only.

That these three sub-groups possess specific differential characters is shown by the following facts: (*a*) the organisms of any sub-group are not agglutinated by the anti-sera of the other two-groups: (*b*) they are not protected against by the sera of other sub-groups: (*c*) they do not absorb from anti-pneumococcus serum II the specific immune bodies of the other sub-groups.

Chickering (1915) went further and demonstrated the specific absorption of immune substances from polyvalent anti-pneumococcus serum, not only by living bacteria, but also by bacterial extracts. He showed that a polyvalent anti-pneumococcus serum may be specifically exhausted of its immune bodies for one of the types of the pneumococcus by the addition of a bacterial extract of the corresponding type, and that the immune substances of the other type remain intact and can be removed subsequently by the addition of the appropriate antigen.

MENINGOCOCCUS AND ALLIED ORGANISMS.

Albrecht and Gohn (1901) were the first to apply the methods of agglutination to the meningococcal group of micro-organisms and showed that it was possible to obtain a specific agglutinating serum for the meningococcus by the intraperitoneal injection of the meningococcal cultures. This work was confirmed and extended by others especially by Lieberknecht (1909) who showed conclusively that, by the employment of serological methods the true meningococcus could be identified and differentiated from the other gram-negative cocci that are frequently found inhabiting the nasopharynx of normal individuals.

The close relationship that exists amongst the Gram-negative group of diplococci is well shown by a study of their morphological cultural and agglutinating properties, which makes them an admirable group for investigation by the absorption method.

Dopter and Koch (1908) showed that anti-meningococcus serum will agglutinate both meningococci and gonococci, and similarly anti-gonococcal serum will agglutinate both gonococci and meningococci. By the absorption method they showed the anti-meningococcus serum

contains a specific agglutinin for meningococci and a non-specific agglutinin for gonococci and that the inverse proposition is true for anti-gonococcal serum. Hence this method demonstrates the fact that these organisms are two specifically distinct germs.

Elser and Huntoon (1909) repeatedly confirmed and amplified Lieberknecht's work and came to the conclusion that gram-negative cocci, having the cultural and fermentative reactions of the meningococcus, are not necessarily that organism. They also imply, although they do not definitely state, that the identification of the meningococcus by means of the agglutination and absorption tests is not always reliable. Elser and Huntoon (1909) further suggested that the term meningococcus was applied to a group of organisms though they did not go so far as to divide that group into definite sub-groups or "types." Tulloch (1917) suggests that their failure to indicate definitely the sub-divisions of the groups may have depended to some extent upon the fact that the sera which they used in their agglutination and absorption tests were of very variable titre, thus introducing possible errors of technique which are difficult to control.

By adopting the absorption test, Darre and Dumas (1914) found a new species of parameningococcus, and came to the conclusion that several varieties certainly exist, just in the same way as there exist several varieties of the paratyphoid bacilli. They state that it is only by the application of the saturation of agglutinins that the differentiation of these diverse species becomes possible.

Dopter and Pauron (1914) applied the saturation test both of agglutinins and precipitins to the differentiation of the meningococcus and parameningococcus.

Dopter and Pauron (1914) also differentiated the parameningococci into three types by means of the saturation of agglutinins, and named these types parameningococcus α , parameningococcus β and parameningococcus γ , respectively.

Gordon (1915) applied the absorption test in order to determine whether the capacity of the meningococcus for absorbing its own agglutinin could be used practically for the purpose of identifying the micro-organisms of the outbreak of that year. The preliminary observations dealt with in this communication appeared to suggest that the capacity of the meningococcus for absorbing its own agglutinin can usefully be employed for identifying that organism. He states that it must be borne in mind that possibly and even probably there are several different strains of the meningococcus capable of producing meningitis,

and that some of these meningococci may not absorb the specific agglutinin of others. How many different strains there are at work at present is a matter of prime importance and further observations are needed for the practical application and limits of the absorption test for the purpose of identifying the meningococcus can be regarded as defined.

These further observations have now been supplied by Gordon and Murray (1915) and by Tulloch (1917). The former observers isolated meningococci from the cerebro-spinal fluid of cases of cerebro-spinal fever, and found that the organisms so obtained could be resolved by the absorption of agglutinin test into four groups, viz. I, II, III, IV. Of the 32 meningococci in question 19 belonged to Group I, 8 to Group II, 4 to Group III and 1 to Group IV. Further, of 9 specimens of gram-negative cocci isolated from the nasopharynx of contacts and suspected cases, and closely resembling the meningococcus in morphological, cultural, staining, and fermentative characters, by the application of the absorption test five were found to be identical with Group II and one with Group I of the cerebro-spinal fluid meningococci.

TABLE VIII.

Type II Serum.

	Agglut. 1/50 for 24 hrs. at 37° C.	Unsaturated				Saturated				Saturated			
		Test coccus added				Homologous coccus added				Test coccus added			
		1/100	1/200	1/300	1/400	1/100	1/200	1/300	1/400	1/100	1/200	1/300	1/400
2 Gar.	-	-	-	-	-	+++	+++	+++	+++	-	-	-	-
11 "	-	-	-	-	-	+++	+++	+++	+++	-	-	-	-
g) Br. F.	(+)	-	-	-	-	+++	+++	+++	+++	-	-	-	-
f) Bow.	-	-	-	-	-	+++	+++	+++	+++	-	-	-	-
h) Cr.	++	-	-	-	-	+++	+++	+++	+++	-	-	-	-
i) Y.	-	-	-	-	-	+++	+++	+++	+++	-	-	-	-
j) Pon.	+	-	-	-	-	+++	+++	+++	+++	-	-	-	-
Control type I	-	-	-	-	-	+++	+++	+++	+++	-	-	-	-
" " II	+++	+++	+++	+++	+++	-	-	-	-	-	-	-	-
" " III	-	-	-	-	-	+++	+++	+++	+++	-	-	-	-
" " IV	++	+	-	-	-	+++	+++	+++	+++	-	-	-	-

By the use of similar methods in the investigation of the meningococci obtained from the cerebro-spinal fluid in 107 cases during the current outbreak of cerebro-spinal fever, Tulloch (1917) found that the organisms described by Gordon as Types I, II, III and IV meningococcus comprise all the organisms responsible for all the cases of cerebro-spinal fever with the exception of three, in which the organism could not definitely be typed, and three others, in which the investigation could

not be completed owing to the organisms having died before the complete series of tests was applied to them. The subdivision of the group meningococci into the types described finds full justification. He calls special attention to the fact that the technique employed was, as far as possible, standardized, and the following is one of the many tables given showing the kind of results obtained.

TETANUS.

Tulloch (1917) in an investigation on the bacteriology of wound infections in cases of tetanus applied serological methods to the differentiation of *B. tetani*. Simple agglutination showed that at least three serological types of this organism exist capable of producing a tetanizing poison. Absorption of agglutinin tests were therefore carried out.

TABLE IX.

Saturation of "U.S.A." Agglutinating Serum.

	Unsaturated serum			Saturated serum, homologous bacilli added			Saturated serum, test bacillus added		
	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400
U.S.A. II	++	++	++	-	-	-	-	-	-
U.S.A. (whole)	++	+	+	+	-	-	-	-	-
T 67 R viii	-	-	-	++	++	++	-	-	-
T 80 B i	-	-	-	++	++	++	-	-	-
R 220 Sp	-	-	-	++	++	++	-	-	-
T 72 B.R.A.	-	-	-	++	++	++	-	-	-

Saturation of "T 67" Agglutinating Serum.

	Untreated serum			Saturated serum, homologous bacillus added			Saturated serum, test bacillus added		
	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400
U.S.A. II	-	-	-	++	++	++	-	-	-
U.S.A. (whole)	-	-	-	++	++	++	-	-	-
T 67 R viii	++	++	++	-	-	-	-	-	-
T 80 B i	++	++	+	-	-	-	-	-	-
R 220 Sp	-	-	-	++	++	++	-	-	-
T 72 B.R.A.	-	-	-	++	++	++	-	-	-

Saturation of "R 220" Agglutinating Serum.

	Untreated serum			Saturated serum, homologous bacillus added			Saturated serum, test bacillus added		
	1/100	1/200	1/400	1/100	1/200	1/400	1/100	1/200	1/400
U.S.A. II	-	-	-	++	++	++	-	-	-
U.S.A. (whole)	-	-	-	++	++	++	-	-	-
T 67 R viii	-	-	-	++	++	++	-	-	-
T 80 B i	-	-	-	++	++	++	-	-	-
R 220 Sp	++	++	++	-	-	-	-	-	-
T 72 B.R.A.	++	++	++	-	-	-	-	-	-

Each serum was absorbed by adding to it emulsions of six organisms—two representatives of each serological type—2.5 cc. of 2000 million emulsion being allowed for each 0.5 cc. of serum. The tubes were incubated for twenty-four hours at 37° C. were then spun and the clear supernatant fluid was used to agglutinate the homologous organism and also the test organism in dilutions of $\frac{1}{100}$, $\frac{1}{200}$, and $\frac{1}{400}$. The results were controlled by reactions with unabsorbed serum in each case.

Table IX giving the results obtained, clearly proves the existence of three types of *B. tetani* serologically distinct from each other.

PLAGUE.

Agglutination and absorption tests have also been employed in the investigation of plague bacilli in Malta by Zammit and Broughton-Alcock (1917), making use of the time measurement slide method of the latter investigator (*vide infra*). Five human cases of plague were studied, one ending fatally, and of 1000 rats examined 15 were found to be infected with *B. pestis*. The human serum agglutinated the *B. pestis* isolated from man more readily than either of two strains isolated from rats and the serum of the fatal case gave a higher specific agglutination action than the others.

Castellani's absorption test was carried out by first determining the degree of agglutinating action present in the sera. The dilution giving complete agglutination in one minute was employed. Three drops of the serum diluted to half this dilution factor was mixed in a sedimentation tube with three drops of the growth formalinized in broth or agar emulsion. At the end of 24 hours at the laboratory temperature it was found that the *B. pestis* isolated from man and rats removed the specific agglutinin previously present for all in human or prepared animal anti-plague serum.

DIAGNOSIS OF MIXED INFECTIONS OF CLOSELY ALLIED BACTERIAL SPECIES PHENOMENON.

Having discovered the absorption of agglutinins as a method of distinguishing between specific and non-specific agglutinins Castellani (1902) then applied it to:

- (1) The sero-diagnosis of mixed infections, and
- (2) The differentiation of closely allied species and types of bacteria.

It now remains to point out the precise manner in which the test may be applied to these two conditions and finally to give a brief description of the technique which is employed in the performance of the test.

THE SERO-DIAGNOSIS OF MIXED INFECTIONS.

The blood of a patient, suspected to be suffering from enteric, contains a fairly large amount of agglutinins for *B. typhosus* and *B. paratyphosus* B. Is it a case of mixed infection typhoid + paratyphoid B? Or is it a case of typhoid with secondary, non-specific agglutinins for *B. paratyphosus* B? Or a case of paratyphoid B, with secondary non-specific agglutinins for *B. typhosus*? Castellani's absorption method will generally enable us to answer these questions, as it will show which are the *specific* agglutinins present.

(1) Let us assume we have had the following results:

After saturation with *B. typhosus*, the typhoid agglutinin and the paratyphoid agglutinin have disappeared completely or nearly so; after saturation with *B. paratyphosus* B, the paratyphoid agglutinin has disappeared completely or nearly so, while the typhoid agglutinin remains in practically the same amount. These results show that the typhoid agglutinin is the specific one, and therefore the case is one of typhoid with non-specific agglutinins (co-agglutinins), for *B. paratyphosus* B. It is not a case of mixed infection of typhoid + paratyphoid B.

(2) Let us assume we have had the following results:

After saturation with *B. typhosus*, the typhoid agglutinin has disappeared or nearly so, while the paratyphoid B agglutinin remains in practically the same amount. After saturation with *B. paratyphosus* B, both the paratyphoid B agglutinin and the typhoid agglutinin have disappeared completely or nearly so. These results mean that the paratyphoid B agglutinin is the specific one and that the case is one of paratyphoid B with secondary non-specific agglutinins for *B. typhosus*.

(3) Let us assume that we have had the following results:

After saturation with *B. typhosus* the typhoid agglutinin has disappeared completely or nearly so—while the paratyphoid B agglutinin remains in the same amount or nearly so; after saturation with *B. paratyphosus* B, the typhoid agglutinin remains practically unchanged. The two agglutinins disappear on saturation with *B. typhosus* and *B. paratyphosus* B. These results show that both the typhoid and the paratyphoid agglutinins present are specific, and that the case is according to all probabilities one of mixed infection typhoid + paratyphoid B.

THE DIFFERENTIAL DIAGNOSIS OF CLOSELY ALLIED BACTERIAL SPECIES AND TYPES.

Suppose we have isolated a bacillus with the cultural and biochemical characters of *B. paratyphoid* B, and that the bacillus is well agglutinated by a paratyphoid B serum: is it *B. paratyphosus* B? Or is it *B. aertryke*, which as is well known has all the cultural and biochemical characters of *B. paratyphosus* B, and is well agglutinated by paratyphoid B serum? Castellani's absorption method will enable us to give a definite answer. If the bacillus we have isolated is really *paratyphosus* B, we shall have the following results: paratyphoid B serum saturated with organism we have isolated will lose, completely or nearly so its agglutinin for *paratyphosus* B, and also its agglutinin for *B. aertryke*. If the germ we have isolated is *B. aertryke*, paratyphoid B serum saturated with the germ we have isolated will lose its agglutinating power completely or very nearly so on *B. aertryke*, but will not lose or only to a slight extent its agglutinating power on *B. paratyphosus* B.

TECHNIQUE.

I. THE SATURATION METHOD.

The test should be carried out by employing the technique described by Taylor (1918) as follows: Take (say) a paratyphoid B serum agglutinating powerfully both the *B. paratyphosus* B and *B. aertryke*, the agglutination titre for both germs being > 1 in 10,000, and proceed as follows:

(1) Dilute the serum with normal saline to have 1 in 50 dilution; put 2.5 cc. of the diluted serum in a sterile centrifuge tube, which label "tube No. 1." Put the same amount of diluted serum (2.5 cc.) in another centrifuge tube which label "tube No. 2."

(2) Scrape off with a platinum wire the growth of 4 or 5 agar slope cultures of *B. paratyphosus* B and add it to the serum in No. 1 tube. Do not wash off the growth with the diluted serum.

(3) Scrape off, with a platinum wire, the growth of 4 or 5 agar slope cultures of *B. aertryke* and add it to the serum in tube 2. Do not wash off the growth with the diluted serum.

(4) After incubating both tubes at 37° C. for two hours centrifuge them until the whole of the bacilli are precipitated and the supernatant fluids are clear.

(5) Pipette off the supernatant fluids into two separate sterile tubes and test the agglutinating action of both fluids up to a dilution of 1 in

10,000 against both *B. paratyphosus* B and *B. aertryke* remembering of course that the serum is already diluted 1 in 50.

The serum before absorption agglutinated both *B. paratyphosus* B and the *B. aertryke* in very high dilution ($> 10,000$). After absorption with *B. paratyphosus* B the titre for both organisms will be practically reduced to nil or nearly so (> 400). After absorption with *B. aertryke* the titre for this bacillus will be practically reduced to nil or nearly so (> 400), while the titre for the paratyphoid B bacillus will be unchanged or only slightly lessened.

Finally, the dosage of bacilli necessary to produce saturation or complete absorption of the homologous agglutinin will vary with the agglutination titre of the serum employed. This is well exemplified in the following table from Bainbridge and O'Brien (1911):

TABLE X. *The effect of absorbing a serum with varying amounts of heterologous bacilli.*

The volume of serum used was constant, namely 2 cc. of 1 in 10 dilution; the amount of bacilli added varied from 2 to 8 agar slopes of approximately uniform size and density of growth.

A *B. suipestifer* serum was absorbed with *B. paratyphosus* B.

Serum	Agglutination limits after absorption	
	<i>B. suipestifer</i>	<i>B. paratyphosus</i> B.
<i>B. suipestifer</i> serum. Original titre	20,000	5000
„ absorbed with 2 slopes	20,000	<100
„ „ „ 4 „	20,000	<100
„ „ „ 8 „	20,000	—

From this and other similar experiments it is seen that the amount of bacilli to be added must vary according to the agglutinating titre of the serum; the higher the titre the greater the quantity of bacilli to be added.

II. THE SUPERSATURATION METHOD.

By supersaturation the principle of Castellani's absorption method is carried one step further.

Harvey and Wood (1911) showed that an excess of micro-organisms other than *B. paratyphosus* A removed or reduced the amount of detectable specific agglutinin for *B. paratyphosus* A present in the serum.

Broughton-Alcock (1917) confirmed and extended this observation and showed that in a similar manner supersaturation of a serum with emulsions of other than the corresponding micro-organisms removes or

reduces the detectable specific agglutinins for *B. dysenteriae*, Shiga, Flexner-Hiss and M. Melitensis. Based on these facts he has evolved a quantitative method which includes in its technique the utilisation of a practically defined amount of micro-organisms and a practically defined agglutination content of the serum.

By the employment of this method he was able to distinguish the specific agglutinin for *B. aertryke* from the agglutinin for a *B. paratyphosus* B strain due to previous T.A.B. inoculation, whilst an emulsion of *B. Gaertner* did not remove either agglutinin.

This method also showed that the specific agglutinin for *B. typhosus* developed in serum in response to anti-typhoid vaccine inoculation might be temporarily increased by inoculation with anti-paratyphoid vaccine. It was also found that specific agglutinins for *B. typhosus* or *B. paratyphosus* B were not reduced by supersaturation with any other than their respective emulsions. This fact permits the use of the supersaturation absorption test to determine whether specific agglutinins for *B. typhosus* or *B. paratyphosus* B are present in a given serum.

In conclusion, having shown what a large amount of valuable work has already been done and how extensive is the field of investigation opened up by the method of absorption of agglutinins, there can be little doubt that this method will be more and more extensively employed by serological and bacteriological workers in the future.

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EXPERIMENTS ON THE EFFECTS OF DUST INHALATIONS.

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(Report to the Medical Research Committee.)

INTRODUCTION.

THIS investigation was commenced, and in the greater part carried through, in Dr Haldane's private laboratory at Cherwell, Oxford. The work was undertaken at his suggestion and I am indebted to him for help and advice both in planning experiments and in discussing results.

Owing to the war the work at Oxford had to be interrupted before its completion and some of the later experiments were carried out in the laboratories at St Thomas's Hospital, London, and I am indebted to the staff there for the facilities afforded to me: Dr L. S. Dudgeon especially who helped me in the microscopical work.

The work was done for, and expenses defrayed by, the Medical Research Committee under the National Insurance Act, with the object of throwing light on the dangers arising from dust inhalations in mining and many other forms of industrial work, and on the conditions under which precautions against dust inhalations are specially necessary.

So many people are, owing to the nature of their employment, compelled to pass a large portion of their time in dusty atmospheres, that this condition and its relation to health has attracted much attention. It may be regarded as established that all forms of dust enter the lungs and that some, such as coal, are relatively harmless, while others, such as flint, are deadly.

Dust rarely kills directly, but by predisposing to pulmonary tuberculosis. On the other hand uncomplicated dust appears capable of producing considerable fibrosis of lung.

There is a vast amount of clinical material bearing on this question, but it is lacking in precision owing to the fact that, under industrial conditions, workers are exposed to mixed dusts and it is difficult to be sure of the offender. There can be no doubt of the deadly character of dust rich in silica: experience in the Transvaal gold mines and in the

potteries is sufficient. But even with silica it is not all plain sailing, for under certain conditions dusts rich in silica appear no more harmful than coal. This comparative immunity might be due either to the conditions of work, physical state of the silica, or the presence of an antidote and, in the event of the latter hypothesis being correct, one might deal with flint by adding the protector. Coal dust on the other hand has its special risk—it is responsible for all the great coal mine explosions. This danger, however, can be reduced to a minimum by mixing coal with inert dusts. The ideal would be to stop colliery explosions by adding flint and to stop phthisis by adding coal, thus holding a tenace over providence.

The particular points selected for study in this investigation are these:

1. A comparison of the behaviour in the lungs of certain harmless and dangerous dusts with a view to deriving a practical means of recognizing what material could safely be used for stone dusting.

2. An attempt to account for the admitted fact that sometimes a dangerous dust may be relatively benevolent, with a view to devising a means of treating the dangerous dust so as to lessen its power for evil.

In addition to the clinical experience that has accumulated there is also much experimental work, of which Arnold's classical investigation is a good instance. Arnold showed that under experimental conditions a variety of dusts enter the lungs and there produced fibrosis. At the time when Arnold performed his experiments there was still some dispute as to whether dust actually entered the lungs; Virchow at first held that the appearances observed in these organs was due to pigmentation and not to inhaled foreign material. Following Arnold most of the workers along these lines have aimed at producing lesions, and the exposures were very severe. The experiments of Professor Beattie¹ were an exception to this rule. He showed that with moderate exposures the shale dust used for stone dusting in collieries is relatively harmless to animals as compared with dust from flint, etc.² In the investigation to be reported, the object has been to ascertain why in industrial practice some kinds of dust are harmless and some dangerous. Hence the exposures have been approximated to those found under working conditions—a running test as opposed to a test to destruction.

¹ *First Report of the Explosions in Mines Committee*, Parl. Paper, Cd 6307, 1912.

² *The Medical Journal of South Africa*, 1914.

The clinical and industrial experience culminating in Dr Watkins-Pitchford's investigations and experiments in South Africa, and the laboratory work bring out the following points:

1. A condition known as pneumokoniosis results owing to dust particles setting up cell proliferation which ends in the production of dense connective tissue. This connective tissue occurs first as nodules and these nodules may subsequently coalesce, obliterating large portions of air-containing vesicles.

2. Sufferers from this condition are peculiarly liable to pulmonary tuberculosis (miner's phthisis, potter's rot, etc.).

3. Some dusts, e.g. soot and coal, are comparatively harmless.

The introduction of the practice of "stone-dusting" into coal mines has made it specially desirable to attempt some classification of dusts into those which predispose to phthisis or other serious lung troubles and those which do not. Certain of these materials have been studied with a view to noting what variations there may be in their action and effects under experimental conditions.

As a start in this work it appeared sound to study the action of certain common dusts used pure and in moderate concentrations. Arnold and others have carried out experiments of this nature: but the severity of exposure was greater than occurs under industrial conditions, and the difference in the reaction of the lung to different kinds of dust was one of degree rather than of kind. In the investigation to be reported the exposures have been short and the thickness of the dust cloud moderate, allowing for the fact that the upper respiratory passages of laboratory animals such as guinea-pigs afford more protection than the corresponding parts in man.

The dusts used have been those arising from coal, shale, flint, quartzite from the Transvaal gold-bearing reef, and the dust obtained from the flues of furnaces driving mining plant, hereinafter known as flue dust. This latter variety has been employed to a considerable extent for preventing explosions in coal-mines. Coal has been selected as the "harmless dust," flint and Transvaal quartz as dangerous dust, and "flue dust" as the unknown for testing.

The dusts so far investigated are:

- (i) Coal dust.
- (ii) Shale dust.
- (iii) Quartz dust from the Transvaal.
- (iv) Flint dust.
- (v) "Flue dust."
- (vi) Pure precipitated silica.

Of these dusts, coal dust and shale dust are known from industrial experience and from the results of Professor Beattie's experiments to be relatively harmless, while the flint dust and that from the Transvaal gold-bearing reef are known to be deadly. Flue dust has not, so far, been studied, but is of interest as it is convenient for "stone dusting." Pure precipitated silica has been used to test the comparative importance of the chemical and physical factors in this undoubtedly dangerous material.

APPARATUS AND METHODS USED.

The materials so far used—the dusts of coal, shale, pure precipitated silica, quartzite from the Transvaal, flint, and that from flues—were studied in the following manner. The different dusts were mounted on slides in the same medium as the microscopical preparations and examined under the microscope directly and with crossed Nicols.

The coal dust, precipitated silica, and shale dust were found to be amorphous; they fractured without the formation of edges, and only isolated particles of shale dust exhibited double refraction. The other three dusts took the form of angular particles with sharp edges, and the overwhelming majority of the particles showed double refraction. It was readily noticed that very small angular particles, though easily visible under quite ordinary magnifications, did not light up on rotation and that particles of many kinds other than silica did light up if of suitable size. These points, of course, are common-places, but in this connection it is of importance to recognize that because a tissue contains particles which exhibit double refraction it does not follow that these particles are silica; and the absence of lighting up is no proof of the absence of small particles of silica. These facts have been insisted on and given great prominence by Dr Watkins-Pitchford in his Lecture on "Occupation Diseases in the Transvaal¹." I am indebted to Dr E. L. Collis for giving me the references to this work. Except for the coal all the dusts in a fine state of division were colourless. This is of importance in connection with their appearance in unstained sections where they ultimately blacken.

Guinea-pigs have been the animals used throughout, and they were exposed to dust in apparatus arranged thus:

A wooden box lined with zinc has in its lowest portion a two-bladed fan *A* driven by an electric motor. The dust to be studied is placed in the box and the fan keeps a cloud of it in suspension. This box has no

¹ *Loc. cit.*

lid but another wooden box fits closely over it. This box is floored with a few strands of wire upon which rest the cages and is closed above by a sheet of glass so that the animals and the thickness of the dust cloud can be observed. Into the side is fitted a small tube whence samples of dust can be withdrawn during the experiment, so that some idea may be formed of the comparative thickness of the cloud.

As it seemed advisable to test the thickness of the dust cloud obtaining in the experiments, a glass tube plugged with cotton wool was connected at one end with the inside of the dusting machine and at the other with a water aspirator containing some thirty litres. The same quantity of dust as that used in the experiments was placed in the machine, and the glass tube, weighed before and after running off the aspirator, gave the amount of dust in thirty litres.

The actual amounts were as follows:

Coal	1.367 grms., say 45 mg. per L.
Shale	1.294 grms., say 43 mg. per L.
Flue dust	0.957 grm., say 32 mg. per L.
Flint	0.827 grm., say 27 mg. per L.

Some rather interesting points came out, notably the great importance of the hygroscopic character of the dust. It had been noticed in the animal experiments that the glass top of the machine was frequently as it were splashed with dust clots which would not shake off, though it brushed off readily enough, and that some dust stuck to the zinc lining of the machine sufficiently firmly to resist shaking though it too would brush clean off. A soft brush or feather mop sufficed without scrubbing. The same facts were observed in some cases in these tests when no animals were used and moisture was reduced to a minimum. Shale dust for instance used fresh from the heater yielded 40 odd mg. per litre, but the same dust run again after being left a few hours in the machine only yielded 22 mg. per litre and spattered the roof and sides with more clots. Coal and shale then, the relatively harmless dusts, give—under these conditions—the thickest cloud. This however is not quite fair: flue dust and flint are less dependent on drying, and with several animals breathing into a confined space they maintain their cloud better. It is interesting to note that dusts which clot in the lungs also clot in the machine.

The concentrations given above are maximal; with several animals in the machine affording a large surface whereon dust can settle the cloud soon thins out unless maintained through a hopper as in the experiments where intense dusting was aimed at.

These clouds are of course far in excess of those experienced in mines and works; but the animals only had from 24 hours to 36 hours all told, and their nasal apparatus is better adapted to deal with the situation.

Sections of the lungs and films from the cut surfaces of the lobes were examined. Mr S. G. Shattock, Curator of the Museums at the Royal College of Surgeons and at St Thomas's Hospital, kindly put at my disposal museum specimens credited to various dusts so that I have been able to compare them with my experimental results.

The "raw" dusts were first passed through a No. 90 sieve to remove coarse particles, and then dried on an electric heater to facilitate their suspension in the air. The readiness with which fine dust takes up moisture and "dumps" varies. All the dusts studied were more or less affected, coal dust to the greatest extent, flue dust to the least. Dust readily deposits on the sides of the box and on the animals and, if a thick cloud is required, it must be frequently renewed or shaken down within range of the fan.

The guinea-pigs were kept in separate cages during the actual dusting and it was necessary to place them in sponge bags as they proceeded to lay the dust by passing incredible quantities of water. It was of interest to note that they rarely passed water in the bags.

REPORTS OF EXPERIMENTS.

I. CONTROL STUDY OF NORMAL GUINEA-PIG LUNGS.

There is considerable variation in, presumably, normal guinea-pig lungs¹.

- (i) Some have a much more congested appearance than others.
- (ii) The amount of fibrous tissue at the root of the lung varies.
- (iii) Pigment may be found under the pleura and in the bronchial glands of young animals born at the laboratory, which is practically in the country.

(iv) While there is much lymphoid tissue scattered through the lungs of normal guinea-pigs, the amount appears to vary and there is no pigment in this situation.

(v) The fibrous tissue is practically confined to the root of the lung and large bronchial tubes and arteries; the general framework of the lobes is very slight and composed almost entirely of elastic tissue.

(vi) Owing to the slowness of the framework the lungs tend to collapse with some completeness when the chest is opened; and for the

¹ Cp. Beattie, *loc. cit.*

purpose of these experiments it was desirable to reduce this collapse to a minimum.

2. TECHNIQUE ADOPTED.

The trachea was tied low down before opening the chest and the "pluck" was removed bodily. One lobe was immediately tied off and used for films and the rest of the tissue placed bodily in the fixative. After two or three hours the lobes were cut in slices and left for three hours more in the fixing solution.

The following preparations were usually made:

- (i) From trachea.
- (ii) At bifurcation of trachea and arranged to include the glands and connective tissue in this region.
- (iii) The lobes were cut so that the sections should pass through the apices.

The routine staining was performed with logwood and eosin and logwood and Van Giesen's fibrous tissue stain. Numerous preparations were treated with Weigert's stain for elastic tissue and with Pappenheim's stain for plasma cells. The typical granular leucocyte in the guinea-pig is acidophilic and I found Biebrich's scarlet very useful for showing up these cells both in the lungs and glands. I used this stain on the recommendation of Dr S. G. Scott, to whom I am indebted for much kind assistance and for putting the resources of the histological department at the Oxford University Museum at my disposal. The films were stained either with Giemsa's stain or some other of the eosinates of methylene blue.

3. PRELIMINARY EXPERIMENTS.

Nine animals were used for preliminary experiments. The object of these was to ascertain the general rate at which dust was taken up, the extent and rapidity of its removal after brief exposures, and the behaviour of the upper respiratory passages. Information was also required as to thickness of the cloud of the various dusts which could be maintained by the apparatus.

Animals were killed with coal gas immediately after two hours' exposure and the heads divided in the sagittal plane. In these cases dust was thickly deposited over the turbinate bones and in all the recesses of the naso pharynx. From this region however it was removed in 36 hours. A certain amount was to be found in the mouth and in the upper part of the oesophagus, but not in the stomach. The efficiency

of the "nasal filter" was shown by the comparatively small quantity to be found in the trachea on naked eye examination.

On microscopical examination hardly any dust was to be found in the lungs after two hours' exposure to a small amount; but after three hours' exposure to a fairly thick cloud it was readily to be seen in trachea, bronchi and alveoli. In these cases coal dust and dust from the Transvaal gold-bearing reef were used. Both dusts were tested with each type of exposure.

In these early cases the dust appeared in free clumps for the most part. In fact only in the coal dust cases was there shedding of epithelium to be made out, though in one of the Transvaal dust cases, killed after 36 hours, there were a few typical dust laden cells to be seen. The lungs were congested, but there was no evidence of broncho-pneumonia. None of the animals appeared any the worse. The glands were free.

4. FIRST SERIES OF EXPERIMENTS.

Six guinea-pigs were exposed for two hours per diem to a moderately thick cloud of dust on twelve consecutive week days, giving 24 hours' exposure in all. Coal dust, shale dust, and Transvaal dust were studied, six animals being used in each case.

Two animals from each series were killed on the completion of the experiment, and one animal at intervals subsequently, so as to provide specimens after 7 days, 3 weeks, 3 months and a year. It was impossible to carry out this plan completely in all cases, as some of the guinea-pigs died. None of the animals appeared to suffer any ill effects as the result of the experiments; and considering the condition of the lungs in many cases this was rather astonishing. As to whether these guinea-pigs had the same capacity for doing work as untreated animals it is impossible to say, but they seemed fully equal to the exigences of a hutch life such as eating and breeding. A few died, but the mortality was considerably less than that which occurred in a Mendelian experiment on a considerable scale which was being carried on simultaneously with animals from a similar source.

(a) *The coal dust series.*

Animals killed immediately at close of experiment. The upper air passages were dusty as in the preliminary experiments but the trachea and bronchi were no more affected to the naked eye than after brief exposures. The lungs were grossly pigmented, and the glands were swollen and more conspicuous than in untreated animals; but though

they showed pigment it was not very marked. On microscopical examination no dust was made out in the epithelium or sub-mucous tissue of either trachea or bronchi. Loose dust and eosinophil cells were snared in the cilia of the cells lining the large air passages; but these cells appeared healthy, and the evidence of bronchitis in the way of increase in goblet cells and infiltration by leucocytes was trifling. The bronchi however were often plugged by masses of cell débris containing dust. There was dust to be made out in the lymphatics but round blood vessels rather than round bronchi. The dust in the lungs was largely intra-cellular, comparatively little being free. Many cells—of large and small endothelial type and dust laden—were to be made out free in the alveoli, but there was also much dust in the fixed cells. There was some cell proliferation but no broncho-pneumonic patches. Comparatively little dust had reached the lymph glands at the root of the lungs and it was mostly in large endothelial cells. Eosinophil cells were not conspicuous.

It was rather surprising to note that the lymphoid tissue scattered through the lung was practically free.

Specimen examined a week after close of experiment.

The differences between this case and the previous one were as follows:

1. The upper air passages were quite clear of dust.
2. Free dust had disappeared from the trachea and bronchi, though plugs of dust-loaded débris were still conspicuous in the latter.
3. There were fewer free dust cells in the alveoli though plenty of dust in fixed cells on the alveolar walls throughout the lung: presumably the continued shedding of these is keeping up the supply of free cells.
4. There are numerous masses suggesting coal dust "dumped" on the alveolar walls, since, on focussing, these masses appear on the surface as opposed to within the cells. The cells seem to take up coal dust with such avidity that they burst and deposit the dust which is moistened and clumped by the pulmonary secretion. This probably facilitates its exit via the bronchi.

5. More dust has reached the bronchial glands, where again one gets this impression of "dumping," though dust cells are present also.

Specimen after three weeks.

There appears to be less dust, the dumps seen in the earlier specimen are less numerous and tend to concentrate round the vestibules. Fewer plugs are to be made out in the bronchi, but dust is now appearing in

the connective tissue round the larger branches at the root of the lung. Eosinophil cells are increased in number in this region and appear in the bronchial epithelium.

The glands are similar to the last case. The dust there does not appear to have increased in amount.

The cell proliferation in the lung has not progressed.

Specimen after three months.

The lung is undoubtedly clearing up. Pigment is to be made out under the pleura, but the lung on section is no longer conspicuously black.

There are now hardly any free dust cells, but a good deal is present in fixed cells scattered about, while concentrations appear round the vestibules.

There are no areas of conspicuous proliferation, though there is some general proliferation.

Dust laden plugs are to be found in the bronchi.

The dust is now plentiful in the connective tissue at the root of the lung and is also to be seen between and probably in the epithelial cells lining the bronchi. It is, presumably, on its way out as it has not been observed in this region before and has only appeared since the accumulation in the connective tissue. It can be traced in the muscular and sub-mucous coats.

The glands at the root of the lung are now less pigmented.

Specimen after ten months.

The lung might almost pass for normal. It is not pigmented to the naked eye save one or two patches under the pleura.

Under the microscope very little dust is to be made out. Even that round the bronchi has almost gone, and the cell proliferation and general richness in nuclei is not marked. Such dust as remains is to be seen in fixed cells, mostly isolated, but there are one or two patches to be made out where cells carrying dust appear to have coalesced and lost their staining qualities.

There is no evidence of increase in fibrous tissue and not the slightest sign of the appearance of nodules in the lung substance.

Summary of coal dust series.

(i) The dust cells, mostly cells derived from pulmonary epithelium, take up coal dust with great avidity and are very readily shed. Dust is seen in fixed cells as well as in free cells but the former are for the most

part isolated and one does not meet islets of dust-loaded cells to any extent.

(ii) The free dust cells appear to disintegrate readily and deposit their contents, which are seen as clumps on the surface of fixed cells.

(iii) There is a good deal of proliferation of cell nuclei, but no inflammatory foci and no fibrosis.

(iv) In cases examined some time after the cessation of dusting the coal dust is most conspicuous in two situations: round the vestibules and in the connective tissue at the root of the lung.

(v) Dust leaves the lung via bronchial tubes as plugs of mucin-containing dust and dust-laden cells. The clumps of dust mentioned are most conspicuous round the vestibules and contribute largely to the plugs.

Dust also leaves via lymphatics. It does not accumulate indefinitely in the lymphoid tissue but passes to the connective tissue at the root of the lung and is thence excreted through the large bronchial tubes.

(vi) With moderate dusting all dust is probably eliminated, and lungs of animals thus exposed might pass for normal after about a year.

(b) *The flue dust series.*

Flue dust, as mentioned before, is a dust composed of colourless particles of angular character, the majority of which show double refraction on examination with crossed nicols.

On examination of the animals killed immediately at the close of the experiment it was at once noticed that the lungs were grossly pigmented to the naked eye, though not as intensely black as in animals exposed to coal dust.

When studied under the microscope the particles of dust showed all variations between colourless and black as coal.

Cell proliferation is much more marked than in the cases exposed to coal.

Free dust cells in the alveoli are not nearly so conspicuous as in the corresponding coal cases, and while individual cells do not carry as great a load the invasion of the fixed cells is far more widespread. As far as can be judged the actual amount of dust that gets into the lung is about the same in the two cases, but as the epithelial cells are less readily shed in the animals exposed to this dust, more is to be found in fixed cells and less in the alveoli and in mucinoid plugs in the bronchi.

As with coal dust, but little dust is present in the large bronchial tubes and none is found in the bronchial epithelium or the sub-mucous tissue.

The connective tissue is practically clear and very little dust has reached the bronchial glands.

Specimen examined a week after close of experiment.

The lung is blacker to the naked eye than in the previous cases.

The cell proliferation has progressed, and is more marked than in the animals examined at the close of the experiment. There is the same scarcity of free cells, and most of the dust is now nearly coal black.

There is no tendency for masses of dust to be deposited as was the case with the coal dust. On the other hand the dust cells tend to aggregate and plaques composed of dust-laden cells are a conspicuous feature.

The connective tissue round the blood vessels contains a little dust and some is leaving in the mucinoid plugs.

The glands are very dark on section: they contain plenty of dust, but it is all intra-cellular.

Specimen after three weeks.

This lung is as dark to the eye as the corresponding coal lung.

Under the microscope the cell proliferation has, if anything, progressed: the dust is black, and there is far more of it remaining in the lung than in the corresponding coal case. The plaques of dust-laden cells are a very conspicuous feature. In the connective tissue the dust is still remote from the root of the lung, and is round the smaller blood vessels.

The glands are dark on section, and contain dust. Plaques are now to be seen in this situation.

Specimen after ten months.

The lung is still dark on section and under the microscope there is still plenty of dust, and cell proliferation is marked; in fact in parts it suggests a chronic broncho-pneumonia. The plaques are still conspicuous, and plasma cells occur in their neighbourhood. No young fibroblasts were definitely made out, and there was no pretence to the formation of fibroid nodules. Dust is scattered about the connective tissue, but it has not collected round the main bronchi and does not appear in the muscular or sub-mucous tissue of the tubes. Plasma cells occur.

The glands are dark and dust-laden. Plaques of dust-laden non-staining cells are numerous.

Considering the state of the lungs it was astonishing that the animals appeared in such robust health.

Summary of the flue dust series.

1. Flue dust blackens in the lung.
2. Cell proliferation progresses for some time after close of experiment.
3. While dust enters to about the same extent as with coal dust it leaves far more slowly.
4. Plaques of dust-laden non-staining cells are a conspicuous feature.
5. The dust cells are less readily shed than with coal dust.
6. The animals suffered no direct ill-effects.

(c) *The shale dust series.*

This dust gave results intermediate between the coal dust and the flue dust. On the one hand the initial catarrhal reaction was not as intense as with the coal, and the rate of elimination was not so rapid: on the other hand there was no plaque formation and the latest lungs examined were almost normal in appearance, though a little dust was still to be found.

This dust also blackened in the lung.

(d) *The Transvaal dust series.*

This dust is notoriously deadly, and it was a matter of some surprise to find that the animals appeared to suffer but little inconvenience. The general course was similar to that with the flue dust. The differences noted were two. Firstly the dust did not collect so readily in the bronchial glands; and secondly, though it turned a pale yellow, it did not blacken so quickly. The lungs were obviously abnormal up to the end: cell proliferation was conspicuous, and the dust was still abundantly present. The lungs however did not appear more damaged than those of the animals exposed to flue dust. No fibrosis occurred.

As far as this set of experiments goes the lung behaves as other tissues exposed to the action of irritants. Cell proliferation occurs; large endothelial and mono-nuclear hyaline cells are conspicuous; and active phagocytosis takes place. Where the dust accumulates the large cells coalesce and plasma cells occur. In no case did this pass on to fibrosis, and polynuclear cells were neither an obvious nor a consistent feature. This last is the only difference made out between the behaviour of the

lung and the behaviour of other tissues in the long list of experiments that have been made on inflammatory reactions.

*Examination of films*¹.

These were prepared by rubbing the fresh cut surface of lung on a clean slide. It was thought that there might be some difference in the cytological reaction to the different dusts. Nothing of the kind was made out. The films were like those which would be obtained from peritoneum under similar circumstances, with the exception that there was no consistent invasion by polynuclear cells. In some cases they were marked; but this had no relation either to the type of dust or the period of the experiment. Dust was found in these cells, but it was not common, and on slides where they were numerous crowds of empty eosinophil cells were to be found surrounding loaded cells of endothelial type. There were numerous un-nucleated fragments containing dust, presumably broken off from the large cells. The blackening of colourless dust is well shown by this method.

5. REPETITION OF THE EXPERIMENTS WITH FLUE DUST, SHALE DUST,
AND TRANSVAAL DUST.

As flue dust had not been studied before and there is a question of using it for "stone-dusting" it was desirable to confirm the previous result. The behaviour of the animals was identical with that in the previous set of experiments.

6. SERIES OF EXPERIMENTS AS TO THE RATE AT WHICH DUST
ACCUMULATED IN THE LUNGS.

Groups of six animals were exposed to the dust as before for two hours per diem for a fortnight and an animal was killed every second day. The results were as follows:

With any dust accumulation begins at once and increases with each dusting; but all the dusts do not behave alike. More dust appears to get into the lungs when coal, flue dust or shale are used than with Transvaal dust or flint.

The fact that the two latter dusts are less readily seen than the former is not sufficient to account for the difference.

With coal dust and shale dust the increase is at first rapid but after about the sixth exposure the rate of elimination appeared to approach the rate of invasion; and lungs after 24 hours were not much more laden

¹ Compare Briscoe, *Journal of Pathology and Bacteriology*, 1907, "Experiments on phagocytic action of alveolar cells."

than after 12 hours. This was not the case with the flue dust or the two crystalline silicas. With these the dust increased steadily, though less rapidly as the experiments went on. These experiments were all carried out with dust clouds of quite moderate intensity.

7. EFFECTS OF INTENSE EXPOSURE TO DUST.

As the work advanced very slowly owing to the length of time occupied by each experiment it was decided to compare the effect of intense dusting for brief periods with the previous experiments. A funnel was arranged to act as a hopper and deliver dust continuously to the box from above and each exposure lasted six hours instead of two hours. Even under these severe conditions the guinea-pigs suffered no permanent ill-effects and began to run about the cage and eat as soon as they were released. In one or two cases where the animals seemed at first to be upset this was probably due to the moist heat of the rubber bags in which they were enclosed. The preliminary experiments showed, as was to be expected, that much more dust entered the lungs under these circumstances; and a series of experiments was carried out with all five dusts, in which groups of animals were exposed to the thick cloud for about 36 hours, spread over six days.

The result was rather unexpected. Nearly all distinction between the behaviour of the different dusts disappeared. At this time the supply of guinea-pigs was rather short and it was only possible to use three animals for each experiment. In each case one animal was killed immediately at the close of the experiment, one was killed after about ten days if available, and a group of five were examined after three months. All the lungs contained great quantities of dust, although, in the case of the coal and shale, and to a lesser extent in the case of the flue dust, considerable quantities had collected in the connective tissue at the roots. There was much congestion, and cell proliferation was marked and widespread. In all cases cell aggregates containing dust were to be seen, and the cell proliferation was especially marked around them, where too the plasma cells could be demonstrated. Definite emphysema could be made out, areas of widely distended alveoli contrasting with the areas of cell proliferations. Even in these cases no formation of new fibrous tissue was to be demonstrated, though as the tissues rather suggested an early diffuse granulomatous condition it might have developed had the animals been preserved longer. While the bronchial glands contained quantities of dust in the case of the coal, shale, and flue specimens, there was not nearly as much to be found where flint

and Transvaal dust had been used. Considering the condition of the lungs it was matter of some remark that the animals should have appeared normal and shown no symptoms of illness.

8. AN EXPERIMENT WITH PURE PRECIPITATED SILICA.

This silica is amorphous when examined under the microscope: there is no double refraction, and it is comparatively soft when chewed. It was of interest to test this dust as it afforded an opportunity of comparing the same material in a crystalline and non-crystalline form. It is known too that in some industries dusts containing a high percentage of silica are harmless; and, as has been suggested, this difference in behaviour may be due to difference in physical state.

The animals were exposed to the dust for two hours per diem on twelve days; and when at the end of that time an animal was killed the lungs were found to be practically dust free. The dust forms a fine powder and, when dry, flies well. I can only account for its rapid disappearance on the assumption that it is soluble.

Mr T. F. Winmill, chemist to the Doncaster Coal Owners, has been good enough to make some tests for me in this direction and finds that 100 cc. of N/5 Na_2CO_3 dissolve 90 mg. in 48 hours at 30° C. The solubility rate depends largely on the fineness of the SiO_2 .

9. ANIMALS WHICH DIED.

A group of three animals may be described which died during the course of experiments, one during an experiment with quartzite, one with powdered flint, and one with flue dust. The first two died on the fourth day, and the flue dust case on the sixth. In each case the appearance of the lung was similar. A considerable amount of dust had reached the alveoli, but not an undue quantity for the exposure. Some of it was free, i.e. resting on the surface of cells as judged by focussing, but most of it was intra-cellular. Very many of the laden dust cells were shed free in the alveoli, and comparatively few were embedded. Much of the dust was blackened. Coarsely granular eosinophil cells were conspicuous in each case; but the outstanding feature was acute oedema of lungs, the alveoli being loaded with an albuminous exudate. The damage appeared to be concentrated on the blood vessels, the connective tissue around them being infiltrated with cells. The muscle was vacuolated and did not stain well, while there were numerous extravasations of red cells through the damaged walls. At the time these sections were being studied occasion had arisen to examine materials from animals

killed by exposure to irritating gases such as N_2O_4 , and the similarity was striking. The outstanding difference was that the lungs from the gassed animals showed gross emphysema with a good deal of ruptured lung tissue; and, of course, dust cells were not conspicuous. One of the dust cases however does show marked emphysema of a portion of the lobe, the rest of which is fluid-laden.

One hesitates to relate these deaths direct to the dust for the following reasons:

1. In each case the animal was one of a batch exposed under precisely similar conditions, and none of the others showed symptoms.

2. Out of the large number of animals dusted only these three cases occurred, and their lungs were not excessively dust-laden.

The appearance of the lungs, however, was not that of the ordinary guinea-pig pneumonia which carries off so many laboratory animals; and it is possible that these were cases of pneumonic infections in lungs irritated by dust. It is hoped to examine the next case from the bacteriological point of view; but at the time these deaths occurred facilities for this work were not available.

It is interesting in this connection to again quote Dr Watkins-Pitchford, who describes under the heading "Acute Pulmonary Silicosis" a type of case reported among the coloured workmen. "The lungs are congested, oedematous and mottled with islands of pigmentation. There is no fibrosis. Microscopically the connective tissue is laden with siliceous particles and the alveoli are distended with serous exudate and catarrhal cells. Such cases are rare and due to inhalation of large quantities of dust over a short period."

10. EFFECTS OF VERY SLIGHT EXPOSURES TO DANGEROUS DUST FOR SHORT PERIODS.

The following experiment was devised to test the ability of the lung to deal with a small but definite invasion by dangerous dust.

Groups of three animals were exposed to small quantities of flint dust, Transvaal dust and flue dust for one hour a day on six occasions. One animal from each series was then killed and examined. In each case dust was readily seen in the lungs, though in small quantities. A second batch was examined at the end of a fortnight. Dust was still present, but there was no general reaction. The remaining animals were killed after six weeks, and the lungs might have passed for normal, though dust could be found on careful search.

11. EFFECTS OF SLIGHT EXPOSURES OVER A LONG PERIOD.

The following experiment was devised to test the influence of extending the period over which a given total exposure was spread. The dusts used were coal, flint, and flue dust, the concentration aimed at being that used in the moderate dusting series. The animals were dusted for half an hour a day for eight weeks, Sundays excepted, thus making up the 24 hours total exposure but in smaller units.

The coal dust animals behaved as might have been expected from series 6. In the animal killed two days after the close of the experiment the lung contained very much less dust than in animals who had received similar total exposure within a briefer period—the last dusting being half an hour instead of two hours was bound to influence the character of the lung in this case. In the animal killed after a fortnight the lung was obviously clearing very rapidly and the rate of elimination must have been not far short of rate of invasion.

In the animals exposed to flint and flue dust no such marked difference was observed as compared with those who had a similar dose within a fortnight, but as far as any contrast was observed the lungs were less affected in this experiment than in the earlier series.

12. EXPOSURE TO MIXED DUSTS.

Two fairly distinct types of reaction having been obtained under the experimental conditions, it was then decided to test the reaction to the two types of dust when mixed.

Two lots of six guinea-pigs were taken. One lot was exposed to flint dust, and the other to a similar quantity of flint dust with coal dust added. Exposures two hours a day for twelve days. The same concentration of flint dust was aimed at as in the earlier unmixed experiments. These experiments gave interesting results: the unmixed flint corresponded to earlier experiments, and went on to plaque formation; while in the mixed dust set the coal produced its usual catarrhal reaction and intense pigmentation of lung, this pigmentation gradually passing off, though not so rapidly as with pure coal. In the latest specimens examined, after nine months, there was still naked eye pigmentation, though slight. The fact that these experiments were carried out in London, while the earlier series were performed at Oxford, was not enough to account for retention of pigment, the unmixed flint series serving as a control. The general condition of the mixed dust lung was different to that of the pure flint. On the one hand the dust

was more conspicuous in the earlier stages, and on the other there was no plaque formation and no signs of fibrous tissue in the later cases. The nine month flint lungs showed plaques, and these plaques showed invasion with fibroblasts as judged by the reaction to Van Gieson's stain. Although the mixed dusts animals exhibited a good deal of dust up to the last the lungs were less loaded than those of animals which had received a smaller total quantity of unmixed dust and, in time, might have practically cleared. In addition to the dust there was sufficient cell proliferation to catch the eye up to the last.

When it was seen that the mixed dusts were behaving somewhat differently to the unmixed another series were started. Mixed flint and coal were given, but the total dust was kept equal to the total flint in the control: half the quantity of flint was taken and the balance was made up with coal. It was thought that under these circumstances as complete an elimination might be produced as with the mild dusting of series 10, although the amount of the flint was much in excess of that used for the earlier animals, and the duration of exposure four times as great.

Neither this experiment nor a repetition was conclusive, as none of the animals lived out the necessary number of months. It was only possible to use four for one set and three for another, and in each case the last animal died before the lungs could have been expected to clear. In two animals, one of which survived for five months and the other for nearly six, the lungs were in each case pigmented; but there was no plaque formation: the cell proliferation was extremely moderate; and the amount of dust small. The disappearance of coal was more rapid than in animals when a greater quantity of flint was used, and, as was only to be expected, there was less flint retained with the reduced dosage.

13. SOME FEEDING EXPERIMENTS.

From time to time various organs other than lung and lymph glands were examined. As already found by Arnold, dust was seen in the cardiac muscle, thymus, spleen and bone-marrow. None was found in the alimentary canal wall, salivary glands, liver, kidney, or pancreas.

With a view to testing the effect of dust by the mouth three animals were fed with Transvaal dust, coal dust, and flue dust mixed with their food. None of the animals showed any ill-effects in the course of an experiment lasting four weeks. At the end of this time they were all killed and the alimentary canal and abdominal lymph glands examined.

No dust was found either in the intestinal mucous membrane or in the abdominal lymph glands.

SUMMARY AND DISCUSSION.

With intense dusting all the dusts used produced much the same effects, both immediate and remote.

With moderate exposures some dusts are much more readily eliminated than others. While coal dust and shale dust enter the lung with great readiness they do not produce, under these conditions, permanent lesions; and the lung might pass for normal after a twelve-month.

Flue dust and crystalline silica are not eliminated with such readiness.

Two methods are available for distinguishing the dusts—perhaps three.

1. Coal and shale are taken up by cells which are quickly shed and consequently do not set up processes which block lymphatics. These cells frequently break down, and masses of dust are to be seen on the surface of the alveolar walls.

Flue dust and the crystalline silica are taken up by cells which tend to remain *in situ* and form plaques, which appear early and persist. They are the only site of fibrosis made out in this investigation.

Dusts which form plaques are not readily eliminated.

2. Rate of elimination is more important than rate of invasion. Dusts which are not readily eliminated after moderate exposures are to be avoided.

3. There are two ways out of the lung: direct via the bronchi in the form of plugs of mucin, dust cells and dust; and indirectly via the lymphatics. These ultimately bring some of the dust to the large bronchi at the root of the lung, and the dust can be traced through the connective tissue, muscular, and mucous coats into the epithelium, whence it is excreted. Colourless dusts become pigmented, and to judge from the data as yet available, the more marked the pigmentation the readier the elimination. It is possible that pigment plays a part in this bronchial excretory process, on the analogy of the lipochromes and the deposit of particles in the skin. The different rates at which dusts reach the mucous coats of large bronchi illustrates their relation to lymphatic drainage.

It is of importance to make out whether dusts observe an "all or none rule" or whether they exhibit gradation.

Flue dust for instance in all these experiments produced permanent

lesions; but a great deal entered the lung and a great deal was eliminated. Specimens taken from animals immediately after exposure to flue dust, Transvaal quartz, and flint, show more flue dust than crystalline silica; but in cases taken later, owing to flue dust elimination being more rapid, the dust remaining is nearly the same in quantity in all three.

Experiments were carried out (series 10) to determine whether these three dusts can be eliminated like coal and shale if the amount of dust in the air be slight and the invasion of the lung moderate. It is worth making great efforts to diminish the dust cloud if there are reasonable grounds for thinking that under these circumstances rate of elimination might approach rate of invasion. If, on the other hand, a considerable proportion of any dust that enters remains, then other methods must be sought.

The result of these experiments was not very encouraging although it was fairly clear that dosage was an important factor. While it is certainly not the case that concentration of dust in the air \times duration of exposure is a constant; still with flue and silica the dose must be very small if accumulation is to be avoided.

More encouraging results were obtained by adding coal. The vigorous catarrhal reaction produced results in the relatively inert dust being carried out in the plugs along with the coal; and these experiments have certainly left the impression that under the conditions described the lungs would practically free themselves from flue or crystalline silica dust if these dusts enter in small quantities only and with coal. The fact that crystalline silica—even when present in considerable quantities—does not always tend to produce grave pulmonary disease, may perhaps be accounted for by assuming that when silica is relatively benevolent there is inhaled together with it some substance that provokes a reaction in the lung with shedding of epithelium and expectoration of débris. If flue dust has been used for stone dusting with impunity it may be due to the presence of coal, since, experimentally, it behaves like the silica group when used pure.

Under the conditions of these experiments:

Dusts that make mischief are dusts that accumulate.

Dusts that are eliminated are dusts that produce a marked initial reaction with much shedding of epithelium.

Dusts that accumulate do not produce so marked an initial reaction, there being much less shedding of epithelium.

Dusts that produce an initial reaction tend to carry out with them the more inert.

A FOOD POISONING OUTBREAK AT BRIGHTON.

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AN outbreak of food poisoning which occurred at Brighton in November, 1917, showed several features of unusual interest which makes its publication of value. While the epidemiological inquiries and pathological investigations were carried out separately a coherent narrative is best obtained by combined presentation. The pathological investigations were carried out for, and at the request of, the Local Government Board.

The outbreak occurred in the Royal Sussex County Hospital which at the time contained 369 residents, 227 being patients and 142 staff. Twenty-eight persons suffered from food poisoning, 24 of whom were patients and 4 members of the staff. Two deaths occurred but both were patients already suffering from severe diseases.

PARTICULARS AND CLINICAL FEATURES OF THE OUTBREAK.

The persons affected were all attacked on November 28th and 29th, the interval between the onsets of the first and last cases being 48 hours. The onset was sudden, the principal features being abdominal pain, vomiting and diarrhoea; headache was frequent but usually not severe. The temperature was raised in practically all cases, at times to over 104° F. Rigor was reported in a few cases.

Incubation Period. If, as supposed, the patients were infected at dinner on the 27th November then the incubation periods (in hours) are as follows: 7, 18, 19, 21, 23, 23, 25, 25, 25, 26, 27, 31, 31, 31, 32, 33, 41, 45, 46, 47, 55, 55.

In this connection the effects of aperients are of interest. Of the 18 persons, who partook of fried fish in Ward D, 10 out of 13 who had no aperient on the evening of the 27th were attacked whilst only 2 of the 5 who had aperients suffered. Unfortunately evidence from Ward V is not available but that from Ward D points to the advisability of the administration of an aperient if food poisoning is suspected.

VEHICLE OF INFECTION.

The abruptness of the outbreak and the other features clearly pointed to food poisoning as the cause, although other possibilities were investigated. It will be unnecessary to detail all the different inquiries made in these directions or as regards articles of diet. The only article of food common to all, or almost all, the sufferers was fried fish while the time of onset pointed to the incriminated food having been taken on the 27th. On that day the patients' midday meal was principally of fish; 122 patients in five wards had fried fish, 70 had steamed fish and 35 had other diets. The 24 patients attacked belonged to two wards (V and D) and 23 of these patients are known to have had fried fish. In Ward D 18 patients had fried fish and 13 of these were infected; in Ward V, 21 had fried fish and 10 of these were infected although less severely than the patients in Ward D. The remaining patients did not have fried fish.

Of the patients in the affected wards 59 per cent. of those who ate fried fish were attacked, while of 14 patients who had not partaken of fried fish but were on other diets only one was attacked.

Although there seems to be no doubt that fried fish was the chief vehicle of infection it may also have been carried by other foods as one nurse (a severe case) and one patient said they had not partaken of fried fish. It has to be remembered, however, that the inquiry as to cause was made on the 29th November and 1st December, and when any large number of persons are affected mistakes in recalling diets are sure to be made, also there is the chance of infection of one food stuff by another, the fish and meat for the day's consumption, both before and after cooking, being in close contact.

BACTERIOLOGICAL EVIDENCE AS TO THE CAUSE OF THE OUTBREAK.

The material examined consisted of blood and internal organs of one fatal case, blood-serum from a number of the cases, excreta specimens from a few cases. Unfortunately none of the fish was available for bacteriological examination.

Fatal case (F. F.). Age 30, a soldier. Died December 8th. *Post-mortem* performed by Dr Galt, Pathologist to the Hospital, 20 hours after death and to whom we are indebted for the following particulars.

The small intestine showed only slight evidences of irritation and contained no blood. From the ileo-caecal valve to the rectum the colon showed acute ulcerative colitis. The mucous membrane at parts was

destroyed, but there was no evidence of extension of the ulceration into the muscular wall. Much bloody mucus was present throughout the colon. There were no other pathological lesions of moment, but the above is of importance in view of our findings since Dr Galt's view is definitely that the cause of death was ulcerative colitis of long standing, and that his death was not directly traceable to the outbreak.

This patient took oxalic acid 5 years previously and had suffered from colitis ever since, and was admitted in June 1917 with intractable diarrhoea with some blood. Later much mucus with a little blood in the stools.

The material received by one of us for examination consisted of a piece of spleen, some heart blood and pieces of the ileum and colon. No organisms of the Gaertner or Proteus groups were isolated from the ileum or colon. From the spleen in pure culture and from the heart blood, mixed with a few *B. coli* and streptococci (both no doubt a *post-mortem* invasion), a bacillus was isolated which for convenience we will call "Brighton." The characters of this Brighton organism were as follows:

A short bacillus with rounded ends, non-sporing, Gram negative but staining well by ordinary dyes. When first isolated showed only moderate motility but active movement after one or two days' artificial cultivation. White, circular, rapidly growing colonies on neutral-red-lactose-bile-salt agar. Uniform turbidity in broth. Bluish translucent growth upon gelatine slope without liquefaction. No indol produced in peptone water media. In litmus milk first some acid production then marked alkalinity. It fails to produce either acid or gas in lactose, salicin, saccharose or raffinose media. In glucose it produced when isolated acid but only a bubble of gas, while in dulcite, mannite and glycerine it produced acid but no gas. Its reactions with other sugars and alcohols were not tested for about two months. It then fermented sorbite and maltose with acid and gas production, produced acid and slight gas in galactose and xylose while it failed to produce either acid or gas in inulin, adonite or amygdalin. Its pathogenicity to rodents was well marked. 2 cc. of a 24 hours broth culture injected intraperitoneally into a guinea-pig (weight 230 grams) killed it in less than 18 hours. The inoculated bacillus was recovered in pure culture from the spleen, liver and heart blood.

The organism as isolated differed from members of the true Gaertner group, such as *B. enteritidis* or *B. paratyphosus* β in its failure to produce gas in glucose (only one bubble of gas after repeated tests), mannite and dulcite.

Excreta from four cases. Unfortunately the samples had to be sent for examination just before Christmas and there was great delay in transmission, only being received four days after collection. In view of this delay and the fact that they were samples collected over three weeks after the attack no conclusions can be drawn from our failure to isolate the "Brighton" organism or members of the typhoid, Gaertner and dysentery groups. The patients having left the hospital it was not found possible to obtain fresh specimens.

Sera from cases. The fact that the "Brighton" organism was isolated from the internal organs of the case by no means proves its association with the disease, especially since this patient was suffering from long old standing colitis with dysenteric symptoms. An extended series of serological tests were carried out to try and clear up this question.

Table I gives the agglutination reactions with the serum of a number of persons who were attacked with food poisoning symptoms, and one (H.) who handled the fish.

TABLE I.

Sera from sufferers from the food poisoning outbreak and from Miss H.

Bacillus tested	F.F.	Col.	W.	B.	C.	P.	O.	H.
<i>B. brighton</i>	100	1000	300	200	200	200	—	100
<i>B. enteritidis</i>	100	1000	300	200	200	300	—	100
<i>B. paratyphosus</i> β	100	100	30	50	50	50	—	30
<i>B. suipestifer</i>	100	100	30	200 (p)	200 (p)	200 (p)	—	—
<i>B. typhosus</i>		300		500	200	300	—	
<i>B. dysenteriae</i> (Flexner)		300		200 (p)	100 (p)	30	—	
„ (Shiga)		—		—		—		

The figures give the limits of positive agglutination reactions.

(p) indicates the reaction was partial with the highest dilution.

— = no reaction; usually in 1 : 30 dilution.

The agglutination results are very interesting but in themselves not conclusive. It will be noted that all the sera except one agglutinate "Brighton" bacillus in at least a 1 per cent. dilution, but on the other hand other bacilli, especially *B. enteritidis* and *B. typhosus*, are equally agglutinated. The question at once arises whether any of these positive results can be ascribed to the effects of inoculation against typhoid, paratyphoid or dysentery fevers singly or combined. Specific inquiry showed that F. F., C. and O. were soldiers and had been inoculated, but P. and B. were civilians, while Col. and W. were nurses in the Hospital, and H. a kitchen maid. None of these five had been inoculated.

TABLE II.

Absorption tests, i.e. agglutination tests with the respective sera after absorption by the bacilli indicated.

		P.			B.			Col.			
		Bacillus	50	100	200	50	100	200	50	100	200
After absorption by Brighton bacillus	Brighton	-	-			-	-		-	-	
	Enteritidis	-	-			-	-		-	-	
	Paratyphoid β	-	-			-	-		-	-	
	Typhoid	-	-			-	-		-	-	
	Flexner	-	-			+ p	+ p	+ p	+	+	+
After absorption by <i>B. enteritidis</i>	Brighton	+ p	-	-		-	-		-	-	
	Enteritidis	-	-			-	-		-	-	
	Paratyphosus	-	-			-	-		-	-	
	Typhoid	-	-			-	-		-	-	
	Flexner	+ p	-	-		+	+	+	-	-	
After absorption by <i>B. typhosus</i>	Brighton	+	+ p	-		+	+		+	+	-
	Enteritidis	+	+	+ p		+	+		+	+	
	Paratyphosus	-	-			-	-		-	-	
	Typhoid	-	-			-	-		-	-	
	Flexner	+ p	-			+	+	-	+	+	+
After absorption by <i>B. paratyphosus</i> β	Brighton	+	+	-		+			+	+	-
	Enteritidis	+	+	+ p		+	+	+ p	+	+	-
	Paratyphosus	-	-	-		-	-		-	-	
	Typhoid	+	+	-		+	+	+	+	+	-
	Flexner	+	-	-		+ p	+ p		+	+	+

Dilutions of 1 : 50, 1 : 100 and 1 : 200 employed.

The agglutination tests are clear evidence of infection and the facts suggest that this was due to infection with "Brighton," the agglutination results with other bacilli being due to the well-known associated agglutinin reactions (co-agglutinins). To settle this point the series of absorption tests set out in Table II were undertaken. The sera selected were cases uncomplicated by any inoculations. Apart from the Flexner results, which are anomalous, the absorption tests show that absorption with either "Brighton" or *B. enteritidis* removes the whole of the agglutinins, while on the other hand absorption with *B. typhosus* or *B. paratyphosus* β , while removing all agglutinins for these organisms largely fails to remove the agglutinins for either the Brighton bacillus or *B. enteritidis*. These results show definitely that the positive agglutination results with these sera are due to infection either with Brighton bacillus or with *B. enteritidis*, and they suggest that these two bacilli are almost identical in their serological behaviour. This important

point was cleared up by immunizing a rabbit with the Brighton bacillus. The serum, withdrawn January 14th, gave the following results:

TABLE III. *Strains tested.*

Dilution	Brighton	H.	<i>B. enteri- tidis</i>	<i>B. para- typhosus</i> β	<i>B. suispe- tifer</i>	<i>B. typhosus</i>	<i>B. dysenteriae</i> (Flexner)
1 : 200	+	+	+	—	—	+	+
1 : 500	+	+	+	—	—	+	—
1 : 1000	+	+	+			+	—
1 : 2000	+	+	+			—	—
1 : 5000	+	+	+			—	
1 : 10,000	— a	— a	—			—	
1 : 15,000	—	—	—			—	

These results show that the Brighton bacillus and *B. enteritidis* are serologically identical, while the positive reactions with *B. typhosus* explain the positive reactions met with in the sera of patients.

THE SOURCE OF INFECTION OF THE INCRIMINATED FOOD.

From the above considerations it is evident that the outbreak was due to infection by a Gaertner group bacillus, allied to but not identical with *B. enteritidis*, and that the vehicle by which the bacillus was conveyed to the sufferers was by means of fish eaten after frying. The next point to determine was the means whereby the fish became infected.

All the evidence is to the effect that the fish as delivered was fresh and in good condition and no objections to it were raised by anyone. This fact, together with the length of the incubation periods, the isolation of a Gaertner group bacillus and the presence of specific agglutinins in the blood, quite negatives any possibility of the outbreak being due to decomposing food.

A second possibility, that the fish was infected with this bacillus on delivery in Brighton from Milford Haven on November 24th, is rendered extremely unlikely by the fact that up to the evening of the 26th it was stored with the fish for sale in the shop, indeed half of the same consignment was sold to private customers and there was no report of consequent illness.

The evidence therefore points to the fact that the place of infection must be looked for either at the fishmonger's or subsequently at the Hospital. The treatment of this batch of fish was as follows: On the evening of the 26th the fish for the Hospital (ling and cod) was sliced by the manager and stored by itself in a sink in the basement of the shop; the ice in which it was stored was broken on the floor in the basement close to all kinds of empties and under the prism-light grating of

the shop entrance through which water percolated at times. In the morning the sliced fish was washed before being sent to the Hospital. At the Hospital the chance of contamination did not appear great, as the fish after delivery in the morning between 9 and 10 a.m. was cooked from 10.30 onwards. Immediately before being cooked the larger pieces of fish were further sliced with a rather blunt knife and each slice was dipped in flour before it was placed in the heated fat in which it was fried. The fat remained unchanged throughout the cooking. The handling of the fish (slicing and flouring) and the cooking were mostly done by a kitchen maid (H.).

It is of considerable interest to note that this particular fish consignment was eaten partly as fried fish, partly as steamed fish. Seventy persons ate this fish steamed and none were affected. Doubtless the temperature of steaming would be sufficient to kill any Gaertner bacilli if present and this would account for the immunity.

On the other hand, if the fish had been infected with this bacillus while at the fishmonger's we should have expected (even with the comparatively cold weather) considerable multiplication and some production of toxins. Gaertner group toxins are notoriously heat resisting and would not be destroyed by the steaming. Such toxins would have produced at least some cases of illness and that none occurred is evidence, though by no means conclusive evidence, that infection was of later date, i.e. probably on the Hospital premises.

BACTERIOLOGICAL EXAMINATION OF THE FOOD HANDLERS.

The sera of all, or at least the most important, of those persons who had handled the fish over the suspected period were bacteriologically investigated. The blood of the man who almost solely handled the fish outside the Hospital and of the cook at the Hospital both gave quite negative results as regards agglutination tests against a number of bacterial strains, but that of the third person, a Miss H., who was kitchen maid at the Hospital gave definite positive results (see Table I). We were fortunately able to obtain a specimen of her excreta on Jan. 8th and from this seven organisms were isolated which were identical with one another and with the Brighton bacillus.

This strain which we call "H" agrees in every particular with the Brighton bacillus and shares its peculiarities, except that one or two of the subcultured colonies gave a bubble of gas as well as acid in mannite media. The agglutination reactions obtained with this bacillus with the

serum of the immunized rabbit (see Table III) are identical with those of the Brighton bacillus and complete the proof as to their identity.

In view of its intrinsic importance it may be pointed out that the rather peculiar deviations of these two strains from the typical Gaertner type make it absolutely certain that these two organisms are identical.

The presence of the Brighton bacillus in the excreta of the kitchen maid H., combined with the positive agglutination reaction, at once raises the question as to whether this girl was the cause of the infection of the fish or was herself a victim of the food poisoning and so became infected with this bacillus. The importance of this point was obvious to us and we made the most careful inquiries to clear it up. We ascertained the following:

(a) Miss H. is positive she did not eat any of the fish and indeed disliked fish. On that day all the fish cooked was sent to the wards and all of it was eaten, none being returned to the kitchen.

(b) She did not suffer from symptoms of food poisoning or illness of any kind during the outbreak. This fact was ascertained by Dr Galt before she was found to harbour the bacilli and was reinvestigated by one of us again after the bacilli were detected.

(c) She had abundant facilities for contaminating the fish if she was a carrier since she actually sliced and floured the fish which proved to be infective.

We are therefore in a position to affirm that the available evidence strongly suggests infection of the food at the Hospital and that the source of infection of the fish was the kitchen maid (Miss H.) who was the one person who especially handled the fish and in whose excreta was found a bacillus identical in all particulars with the bacillus causing the outbreak.

THE SOURCE OF INFECTION OF THE FOOD CARRIER.

We next endeavoured to carry our inquiries a step further and ascertain when and how Miss H. became infected and a carrier. In this inquiry we met with but little success. This girl was only 17 at the time of the outbreak, and since she left home, a period of two years, had been in service at the Hospital. She had not been off duty except for the usual holidays and neither she nor her mother could remember any illness from which she had suffered.

If, as we conclude, she had been a carrier for some time it is interesting, but not remarkable, that she should not have caused any other outbreaks in view of her favourable opportunities to infect food. This

is probably explainable from the well-known intermittency of infection of carriers, while a large part of the food she handled was cooked before use. Further of course a carrier is only likely to infect when the bacilli have gained access to her hands from accidental or uncleanly self-infection and this may have been a matter of great rarity in this case.

REMARKS AND CONSIDERATIONS OF A NUMBER OF POINTS
OF SCIENTIFIC INTEREST.

(1) The bacillus isolated while clearly of the Gaertner group when isolated did not agree in its cultural characters with any known food poisoning strain. The differences were, however, of degree only, did not involve any added characters and were merely in the direction of diminished fermentation power. Thus the three substances, glucose, mannite and dulcitol, were split up with only acid production but not with the formation of gas also. There was however evidence of very slight gas production and in view of this and for the above reasons we considered that the cultural differences were probably due to transient retardation of fermentation powers. Some mutation of characters is a well-recognized property of this group.

Our surmise was confirmed as after artificial cultivation in litmus milk and other media for several weeks and then replating and retesting moderate gas production with glucose and dulcitol and well-marked gas production with mannite were obtained in both the Brighton and H. strains.

At the same time it is of great scientific interest to note this abnormality of characters when isolated, especially as it was equally well marked in the carrier strain and in the bacillus recovered after passage through a guinea-pig.

(2) The fatal case investigated (F. F.) was ill at the time of the outbreak with colitis and therefore the bacillus isolated could not be accepted at once as having anything to do with the outbreak. This patient not only ate the suspected fish but had a rise of temperature and other symptoms, coincident with the other cases. His blood gave a well-marked reaction to the Brighton bacillus and also to *B. enteritidis* (see Table I). The other facts prove that this bacillus was not one peculiar to and associated with his condition of colitis but was the cause of the outbreak.

One other patient who ate the fish and had symptoms died and *post mortem* his death was found to be due to malignant ulcerative colitis. This case was not bacteriologically investigated.

The fact that the only fatal cases suffered from inflammatory conditions is of especial interest. The ulcerative bowel condition would predispose at least to infection and probably to a fatal infection. Meyer¹ in America has quite recently reported a case which bears upon this point. In this case a young man became infected with *B. enteritidis*, recently isolated from a calf dysentery case, used for feeding calves in an experimental investigation upon calf dysentery. A number of other laboratory workers were equally exposed to infection, the material being handled in a very careless manner, but he only was attacked, evidently because at the time he was suffering from mucous colitis.

(3) The considerable degree to which the blood of the affected persons also agglutinated *B. typhosus* and other strains, and the fact that some of the patients were inoculated soldiers, added a complication which necessitated the use of absorption tests to clear up the matter. The investigation very clearly shows the value of these absorption tests.

(4) Food poisoning outbreaks definitely traced to human carriers are extremely rare. In several Continental outbreaks this source has been suggested but for none of those which we have been able to read is the proof established or even more than a reasonable possibility.

In the 79 outbreaks in Great Britain reported by one of us² to the Local Government Board, in only one outbreak (Wrexham 1910) is a human carrier advanced as the cause and source of infection. In this case the evidence is inconclusive owing to an organism described as *B. paratyphosus* β .

In the Brighton outbreak the bacillus isolated is clearly a slightly abnormal strain of *B. enteritidis*, an organism which only sets up food poisoning outbreaks and never long continued infections such as paratyphoid fever or enteric fever. It is not a natural inhabitant of the human or animal intestine and its presence there can only be ascribed to an infection with food containing these organisms. That a food poisoning outbreak should be traced to a human carrier of this organism is therefore both a unique and a specially interesting fact. We know of no other definite case in the literature of food poisoning.

Its importance made us especially careful to satisfy ourselves that Miss H. was a carrier and not infected during the outbreak. Since the bacilli were only looked for and found after the outbreak we cannot express absolute certainty as to the matter, but the evidence we have

¹ *Journal of Infectious Diseases*, 1916, Volume XIX, p. 700.

² Savage, 1913, Report to the Local Government Board on Bacterial Food Poisoning and Food Infections.

adduced, we think, very strongly favours the view that she was an actual carrier.

The blood of this girl H. still gave a well-marked positive agglutination reaction with "Brighton" bacillus in 1 to 50 dilution and an indefinite reaction in 1 to 100 dilution on both Feb. 13th and March 22nd, 1918. We failed however to find this bacillus in her excreta in specimens collected on both these dates.

(5) We have no difficulty in explaining why only a portion of the consumers of the fried fish were attacked as doubtless the cooking was sufficient to sterilize the fish in most cases. The most difficult part of the outbreak to explain is the survival of any bacilli after the cooking to which they were exposed. The length of the incubation period and other facts make it evident that the infecting bacilli did survive and it is not an outbreak due to heat resisting Gaertner group toxins.

We have been able to elicit a few further facts which bear upon this point. It has already been mentioned that the consumers of the steamed fish and those of fried fish other than to Wards V and D escaped. It is evident therefore that there must have been some factor or factors which prevented efficient sterilization of these particular batches of fish. We have ascertained that on the 27th the frying fat did not cover the fish slices as usual and in addition the cooking was hurried because of late delivery. The fish for Ward D, in which the most severe cases occurred and the greatest percentage were infected, was cooked first and was kept warm on a hot-plate for over an hour, thus encouraging multiplication of any bacilli not destroyed in the cooking, whilst the fish served to Ward V was cooked last and in a hurry, being much underdone, the patients having difficulty in separating the flesh from the bones.

While the temperature of cooking of fried fish is high it is very superficial and these factors must have been sufficient to prevent the killing of all the bacilli.

We are indebted to Dr Galt, Pathologist, and to the resident medical staff of the Hospital, for the pathological and other particulars of the cases and inquiries made amongst the Staff, to Dr Harriette Chick of the Lister Institute for kindly testing our strain upon a number of sugar-alcohols which we had not at our disposal, and to Dr MacFadden of the Local Government Board for permission to utilise the pathological data contained in our report to the Board.

THE STANDARDIZATION OF KILLED AGGLUTINABLE CULTURES OF *B. DYSENTERIAE*.

By A. D. GARDNER, M.D., F.R.C.S.

*(From the Standards Laboratory, Department of Pathology,
University of Oxford.)*

(Report to the National Medical Research Committee.)

(With 2 Charts.)

Up to the present the value of the agglutination test in the diagnosis of bacillary dysentery has not been finally decided. Very numerous have been the attempts to settle the question, and as various have been the methods employed. It is more than probable that the variety and lack of uniformity of technique have been largely responsible for the wide differences in the conclusions arrived at by different experimenters.

The conviction that both improvement and, above all, uniformity of method are essential to the settlement of the question led Prof. Dreyer early in 1915 to consider the possibility of applying to cultures of *B. dysenteriae* the process of standardization that he had already put into use for the preparation of Agglutinable cultures of the Typhoid-Paratyphoid group of bacilli. At his instigation, and largely under his direction, I started the investigations set out in this paper, the practical result of which has been the issue of standard agglutinable cultures of three varieties of *B. dysenteriae* in Aug. 1916, by the Standards Laboratory on behalf of the National Medical Research Committee.

REASONS FOR STANDARDIZATION OF TECHNIQUE.

The following factors are the most important of those that are known to affect the degree or intensity of the agglutination—reaction given by a serum of a particular strength.

- (1) Time.
- (2) Temperature.
- (3) Number or mass of the bacilli on which the serum acts.
- (4) The sensitiveness of the bacilli to agglutination.

Of less fundamental importance, but none the less exercising a very appreciable effect are:

(5) Motion or absence of motion of the fluid.

(6) Area of the surface with which the fluid is in contact.

Further, the recorded result will vary according to the method of observation adopted (e.g., microscopic or macroscopic) and in the arrangement of the light for reading. And, again, the precision of the result will depend upon the fineness of gradation of the dilutions employed.

It is evident that, in the absence of any recognized uniformity of method, uniformity of results is impossible. If one and the same serum be tested by two methods, e.g.,

(1) With a thick suspension of bacilli, at room temperature, readings taken at the end of 30 minutes microscopically.

(2) With a thin suspension, at 50° C., readings taken after 4 hours macroscopically.

The "titre" of the serum by method (1) may work out as one in 500; by method (2) as one in 5000.

Now there is little difficulty in determining what are the best conditions of time and temperature for the agglutination of any group of bacteria. The temperature at which the velocity of the reaction is greatest is clearly the ideal, and in this respect the dysentery bacilli behave in the same way as those of the enteric group, viz., a temperature of 50°-55° C. gives a much higher velocity of reaction than lower temperatures. The best temperature being determined, it remains to fix the period of time required at that temperature for the reaction to arrive approximately at its end-point. In the case of the typho-paratyphoid bacillus two hours has been found sufficient, but our experience with the dysentery bacilli confirms the observations of others that a longer period of time is required for their complete agglutination. As a result of many experiments four and a half hours at 50°-55° C. was fixed upon as the shortest time in which the reaction becomes sufficiently complete for the purpose of taking trustworthy readings.

It is well known that the number or mass of bacteria upon which the serum has to act is a factor of great importance (Gruber 1897, Winterberg 1899, Sacquépée 1901, Dreyer and Jex-Blake 1905). Great variations of titre may be found for a particular serum with bacterial emulsions of varying thickness. Constant results therefore can only be expected either when emulsions of constant thickness are used or when the variations of agglutinability of different emulsions due to this factor are determined by experiment and included in a numerical

reduction-factor which expresses the true relative agglutinability of each emulsion.

Working with formalized broth cultures of bacilli of the typho-coli group Dreyer found that by far the best readings were obtainable with relatively thin emulsions. For with these not only is apparent end-titre of a serum greatly raised, but also it is possible to gauge the precise degree of agglutination in a given tube according to the size of the flocculi suspended in the fluid. With such a method of reading a number of serums which do not differ from each other in strength by more than 10 %, if tested under precisely the same experimental conditions, may be differentiated from one another with certainty, and that without employing any finer gradation of serum-dilutions than is used in the ordinary clinical test.

The optimum density for agglutinable cultures of *B. dysenteriae* is rather greater than for *B. typhosus*, *B. paratyphosus* A and B and *B. coli*, for the flocculi formed by the former group are always small, and they may be too small for good readings if the emulsion is very thin.

The standardization of the thickness of the emulsions used in all my experiments was done by the method described recently by G. Dreyer and the writer (1916).

The complete flocculation of dysentery cultures by a serum is assisted by movement or circulation of the fluid. This is also true of other bacilli, but it is of more importance in the case of *B. dysenteriae* owing to the slowness and fineness of the flocculation. Continual movement of the fluid in the agglutination tubes may be obtained by incomplete immersion of the tubes in the water of the water-bath, for the heated lower part of the fluid is continually rising, and the cooler upper part continually falling to the bottom. If two tubes be taken, each containing precisely the same mixture of serum and culture, and one of them be fully immersed (i.e., so that the whole column of fluid is beneath the surface of the water), and the other be only half-immersed: at the end of a given period of time the agglutination in the half-immersed tube will be found to be in advance of that in the fully-immersed tube. The difference, expressed numerically, is usually about 10 %. The differences in a series of tubes between "total," "standard" and "trace," and the finer intermediate gradations of flocculation are more constantly clear and easy to read when the fluid has been kept in motion by incomplete immersion.

Any standard technique must of necessity include the use of tubes of a standard size and shape. For the velocity of the reaction is

distinctly influenced by the size and shape of the tube. The narrower the tube in which a volume of mixture is contained, the further will the reaction have progressed in a given time. In general it appears that within certain limits the greater the surface with which the fluid is in contact, the more rapid and complete is the agglutination.

The factors in agglutination-technique with which I have dealt so far are all easily controllable. Evidently the best technique is that which embodies the optimum temperature and time, the best density of emulsion for high sensitiveness and accuracy of readings, circulation of the fluid, and a uniform and suitable size and shape for the tubes. But there is one more factor which is of greater immediate interest than any one of the above, because it is far less easy to control—and that is the specific sensitiveness or degree of agglutinability of the bacillary emulsions.

If a number of emulsions of undetermined but approximately similar thickness be made from a number of subcultures of one and the same strain of a bacillus, each emulsion will have its own specific degree of agglutinability, which may be greater than, equal to or less than the mean agglutinability of the batch. The variation from the mean may be so considerable that two successive emulsions differ quantitatively to the extent of 100 % or more. If all the emulsions be made of precisely the same thickness, the variation in agglutinability is diminished, but it remains so considerable that it is impossible to devise a quantitatively accurate technique without controlling this variable factor. The necessity for this has long been recognized, by scientific workers on agglutination (Madsen and Jørgensen 1902, Dreyer and Jex-Blake 1905), but a large number of clinical pathologists have, until very recently, been content to work with uncontrolled and variable cultures. Dreyer (1906) elaborated and perfected a technique for clinical typhoid and paratyphoid agglutination tests, in which all the optimum conditions for the reaction were embodied, and the variable factor of the agglutinability of emulsions was fully controlled by the comparative standardization of all emulsions against an original arbitrarily chosen standard emulsion. This entailed the introduction of the agglutinin unit, and the reduction to units of readings first expressed as dilutions.

The essential qualities required in an emulsion that can be employed in this manner are: stability and durability, a sufficiently high agglutinability, and freedom from any tendency towards spontaneous agglutination.

The main object of my investigation was to discover whether emul-

sions of this kind can be made from cultures of the different groups of *B. dysenteriae*, and whether these bacilli behave in the same way as the typhoid-coli group, or in a different way.

Since Dreyer's investigations had proved the superiority of broth cultures over saline suspensions in the case of the typhoid group, it was natural to expect a similar superiority when other, but not widely different, bacilli are in question. From the start, therefore, I have experimented with broth cultures, and I have only used saline suspensions in some comparative experiments with a microscopic technique.

An attempt has recently been made by O. Schiemann (1916) to prepare a stable emulsion of dysentery bacilli (*Ruhrdiagnosticum*) for agglutination. He treated saline suspensions of the bacilli with heat of various degrees and with a number of chemical substances, including formalin, phenol, thymol and chloroform, but he failed to prepare a satisfactory emulsion by any of these means. All emulsions began sooner or later to agglutinate spontaneously. His failure must be attributed to the use of saline suspensions, since by almost identical procedure it is quite easy to make from broth cultures perfectly stable and satisfactory standard dysentery cultures.

EXPERIMENTS WITH FORMALIZED BROTH CULTURES OF DYSENTERY BACILLI. TECHNIQUE OF PREPARATION.

The method of preparation was that recommended by Prof. Dreyer for the preparation of standard agglutinable cultures of typhoid and paratyphoid bacilli and described on a leaflet which is issued with these cultures on behalf of the National Medical Research Committee. The leaflet is reproduced here. Paragraphs 1 and 2 deal with the preparation of the cultures (see also Dreyer, 1906) and the method of opacity-estimation and dilution to a constant opacity (see also Dreyer and Gardner, 1916). In all opacity tests and in a large proportion of the earlier agglutination experiments I made use of graduated pipettes and dwarf test-tubes. But later on I adopted the drop measuring technique almost exclusively, on becoming satisfied that it is no less trustworthy, and more economic of material.

DIRECTIONS.

PREPARATION AND STANDARDIZATION OF AGGLUTINABLE CULTURES.

B. typhosus, *B. paratyphosus* (A and B), *B. dysentericus* (Flexner, Shiga, and Y),
B. enteritidis (Gaertner), *B. coli*, and *Vib. cholerae*.

1. *Preparation.*

The bacillus is grown for 24 hours at 37° C. in ordinary *veal* peptone bouillon¹ in large Erlenmeyer flasks partly filled (1 litre of bouillon in a one and a half litre flask).

Before use the flasks of bouillon are sterilized in the autoclave at 115° C. for *not more* than 15 minutes, and then tested for sterility by incubation at 37° C. for 48 to 72 hours.

They are inoculated with a few drops each from a 20 to 24 hours old bouillon culture of the bacillus (*B. typhosus*, *B. paratyphosus*, etc.).

The culture used should be one which has been subcultivated daily in bouillon for one or two weeks (or longer). This continued subcultivation has the effect of increasing its agglutinability and diminishing any tendency to spontaneous agglutination.

At the end of 20 to 24 hours' growth at 37° C. the flasks are well shaken, and to each is added 0.1 per cent. (1 e.e. per litre) of commercial (40 per cent.) formalin. They are again shaken and placed in a *cold chamber* in the dark at about 2° C.

At intervals on the same day and on subsequent days for four or five days the flasks are again thoroughly shaken and *replaced at once in the cold chamber*.

After three or four days they will be found to be absolutely sterilized. Should it happen that the bacterial suspension is not entirely homogeneous it may be shaken for some hours in a mechanical shaker, or may finally be filtered through sterile cotton-wool.

2. Standardization.

The process of standardization consists (a) in making up the killed culture to an *opacity* as nearly as possible identical with that of the standard agglutinable culture, (b) in measuring its *agglutinability* as compared with the standard agglutinable culture by the use of standard serum.

Dysentery cultures should after dilution to standard opacity be left to stand in the cold store for one or two months before they are standardized for agglutinability and used for agglutination tests.

(a) The *degree of opacity* (turbidity) of the new culture as compared with that of the standard culture is determined as follows:

Take two stands and place eleven agglutination tubes in each. The tubes must be chosen so as to be of very nearly equal internal diameter (for this purpose some kind of a gauge is almost essential), and their surface must be devoid of scratches.

Prepare

- (1) a few c.e. of a 1 in 2 dilution of the new-killed culture.
- (2) " " " " " standard culture.

¹ The bouillon is titrated against phenolphthalein, and two-thirds of that amount of sodium hydrate, which would render it neutral to phenolphthalein, is added before the final boiling and filtration.

With the dropping pipette (held vertically) measure out

			Drops of water	Drops of culture	
Into tube 1 of each stand			0	20	
”	2	”	4	16	} Culture full strength
”	3	”	8	12	
”	4	”	10	10	
”	5	”	12	8	
			<hr/>	<hr/>	
”	6	”	6	14	} Culture diluted 1 in 2
”	7	”	8	12	
”	8	”	10	10	
”	9	”	12	8	
”	10	”	14	6	
”	11	”	16	4	

One series of tubes receives the new-killed culture, the other the standard culture. Mix the fluids in the tubes well, and take comparative readings with artificial illumination against a dark background. A frosted electric bulb with a large opaque shade and a piece of dull black board or paper propped up behind gives the best results.

Hold up the tubes to be compared against the edge of the lamp-shade, which is arranged so that the source of light is just hidden from the eye, while it illuminates the particles suspended in the fluids. A darkened room renders the reading easier and more accurate. The outside of each tube must be cleansed and wiped dry before a reading is taken.

A tube is chosen at random from one series, and it is compared with the tubes of the other series in succession, until one is found to match it in opacity. The identity of these two tubes is recorded as the first reading.

If the tube chosen fails to correspond exactly with a tube in the other series, but is seen to have an opacity intermediate between the opacities of two adjacent tubes, the reading is recorded thus:

e.g., Series I tube 3 = Series II tubes 4-5.

Several tubes in one series are thus matched in succession with tubes of the other series, so as to obtain a number of separate readings.

Suppose that two of the readings are:

	Standard cul- ture series		New culture series
(1)	Tube 5	=	Tube 9.
(2)	Tubes 2-3	=	Tube 6.

(1) Reference to the table shows that tube 5 contains 8 drops of undiluted culture, tube 9 contains 8 drops of a 1 in 2 dilution, i.e., 4 drops of undiluted culture.

Therefore 4 drops of the new culture give the same opacity as 8 drops of standard culture, i.e., the new culture is twice as opaque as the standard.

(2) Tube 6 contains 14 drops of diluted, or 7 drops of undiluted culture. Tube 6 in the new culture series falls midway between tubes 2 and 3 of the standard series which contain 16 and 12 drops of full strength culture respectively. It therefore corresponds with 14 drops of standard culture.

Again it is found that the new culture has twice the opacity of the standard.

In practice the readings are found to vary a few per cent. on either side of the mean, and therefore it is best to take at least five readings and calculate an average. From these data the degree of dilution necessary to bring the new culture to standard opacity is determined.

E.g., in the example quoted the new culture must be diluted one in two.

The diluting fluid used is normal saline solution to which 0·1 per cent. of commercial formalin has been added.

If a high degree of accuracy in the opacity-estimation is required, the measurements may be made with a graduated 2·0 c.c. pipette into larger tubes, tenths of a c.c. being substituted for drops.

(b) To measure the *agglutinability* of the killed culture thus diluted proceed as follows:

Take two stands¹ and place 12 agglutination tubes in each. Prepare (1) a dilution of standard agglutinating serum of such strength that each cubic centimetre contains from four to eight standard agglutinin units, and from this prepare (2) a second dilution of half that strength.

With the pipette measure out

			Drops of normal saline solution	Serum dilution		
Into tube 1 of each stand			0	10 drops of dilution 1		To each tube of one stand is added 15 drops of <i>Standard Agglutinable Culture</i> , and to each tube of the other stand 15 drops of the <i>killed culture</i> under standardization.
" 2	"		2	8	"	
" 3	"		4	6	"	
" 4	"		5	5	"	
" 5	"		6	4	"	
" 6	"		3	7 drops of dilution 2		
" 7	"		4	6	"	
" 8	"		5	5	"	
" 9	"		6	4	"	
" 10	"		7	3	"	
" 11	"		8	2	"	
" 12	"		10	0	"	

At each stage of the procedure the pipette is carefully washed and dried out with successive quantities of absolute alcohol followed by successive quantities of ether.

The stands are placed for two hours (in the case of dysentery cultures 4½ hours) in a water-bath at 50° C.-55° C., then allowed to stand for 15 to 20 minutes at room temperature and a reading subsequently taken by selecting in the series made with standard agglutinable culture the tube which exhibits standard agglutination (the highest dilution in which marked agglutination, without sedimentation, can be detected with the naked eye), and ascertaining which tube in the other series shows the same degree of agglutination. This comparison is repeated and made more exact by selecting in succession from the first series one or two other tubes which exhibit less than standard agglutination and similarly matching them in the second series. Should the tube be the same in each series the agglutinability of the killed culture

¹ Stands, dropping pipettes, agglutination tubes, etc., can be obtained from Messrs Baird and Tatlock, Hatton Garden, E.C., or from Messrs R. B. Turner and Co., Eagle Street, Southampton Row, W.C.

is clearly equal to that of the standard. If not the same, the degree of agglutinability of the killed culture is now readily determined.

Thus suppose that tube 5 in the standard series corresponds to tube 2 in the other series. The standard agglutinable culture is twice as agglutinable as the killed culture under standardization, since only half the quantity of serum has been required to agglutinate it to the same degree.

Hence, if any given serum presented for examination is found to agglutinate this particular killed culture in a dilution of, say, 1 in 500, then 500 multiplied by 2 and divided by the figure given on the label of the standard agglutinable culture is the number of *standard agglutinin units*¹ in 1 c.c. of the serum examined.

Or again, if the killed culture were, say, 1.3 times as agglutinable as the standard agglutinable culture, then, in the same example as above, 500 divided by 1.3 and again divided by the figure given on the label of the standard agglutinable culture is the number of *standard agglutinin units* in 1 c.c. of the serum examined.

In order to increase the accuracy of the standardization the test of sensitiveness to agglutination should be repeated *ab initio* two or three times, and an average taken of the readings thus obtained.

Readings should always be made by artificial light against a dark background.

*From the Department of Pathology, University of Oxford,
on behalf of the National Medical Research Committee.*

TECHNIQUE OF AGGLUTINATION TESTS.

In all essential points the technique followed paragraph 2 (b) of the reprinted leaflet. When graduated pipettes and larger volumes of fluid were used, the relative volumes of the different constituents were nevertheless identical with those given in the table. The actual quantities used in the graduated-pipette technique are—Serum (in c.c.) 1.00, 0.80, 0.60, 0.50, 0.40, 0.35, 0.30, 0.25, 0.20, 0.17, 0.13, 0.10, 0.00. Saline is added to each tube to make a total volume of 1.0 c.c. Then 1.5 c.c. of culture is added to each tube. There is one more tube in this series than in the drop-pipette series, since fractions of 0.1 c.c. can be measured, whereas fractions of a drop can not. The additional tube increases the accuracy of readings when the end-point happens to fall among the higher dilutions of the series. When two or more emulsions of a bacillus were being tested against a serum or against each other, a sufficient quantity of the desired serum-dilution for the whole experiment was always made up, and a number of rows of tubes, one for each emulsion, were filled

¹ The *Standard Agglutinin Unit* is that amount of agglutinating serum which when made up to 1 c.c. volume with normal saline solution causes standard agglutination on being mixed with 1.5 c.c. of a particular standard agglutinable culture and maintained at 50° to 55° C. for two hours (dysentery cultures 4½ hours) in a water-bath followed by 15 to 20 minutes at the room temperature.

with the graduated quantities of the serum-dilution and then with the various emulsions. All were subjected to precisely the same conditions of time, temperature, etc., during the course of the experiment.

READINGS AND CALCULATION.

The system of reading degrees of agglutination followed in these experiments has been used by Prof. Dreyer for many years, and was described in a recent paper by Dreyer and Inman (1917). When properly prepared and formalized cultures are used whether of the enteric or of the dysentery group of bacilli, certain degrees of agglutination are easily recognizable in the neighbourhood of the end-point of the reaction, viz., *Total agglutination* (T), i.e., sedimentation of the flocculated bacilli, and clearing of the supernatant fluid; *Standard agglutination* (S), i.e., well-developed flocculation, the clumps all remaining suspended in the fluid; and *Trace* (Tr), i.e., the least degree of clumping visible to the naked eye. If a long finely graded series of dilutions of a serum be made to act upon a standard culture, each of the above-mentioned degrees of agglutination will be found, together with a number of intermediate degrees. Among these it is easy to recognize *Total minus* (T -), *Standard plus* (S +), *Standard minus* (S -), *Trace plus* (tr +), *Trace minus* (tr -); and for a doubtful reaction *Query trace* (? tr).

From a very large number of records of finely graded agglutination tests of cultures of the typhoid-coli group, it was possible for Prof. Dreyer to work out a reduction table for calculating the numerical value of standard agglutination from any reading between total and nothing.

For instance, if 100 parts of a serum give exactly "standard agglutination," 1.47 parts of the same serum will give "total," and .68 part will give "trace."

The full table of readings, with their reduction values, is as follows:

Reduction Table.

TOTAL	T	1.47
Total minus	T -	1.29
Standard plus	S +	1.13
STANDARD	S	1.00
Standard minus	S -	0.88
Trace plus	tr +	0.77
TRACE	tr	0.68
Trace minus	tr -	0.60
Query trace	? tr	0.53
Nil	0	0.46

The great value of this table lies in the fact that it enables us to calculate the serum-quantity or dilution that will give standard agglutination although we have not actually a tube in the series which gives a "standard" reading, e.g., if a dilution of 1/50 of a serum gives a reading of "tr +," by reference to the table, we can see that standard agglutination will be given by a dilution of

$$\frac{1}{50} \times \frac{1.00}{0.77} = \frac{1}{38.5}.$$

This table, though calculated for the typhoid-coli group of bacilli, is fully applicable to dysentery-agglutination, provided that a considerably finer flocculation be taken as "standard agglutination" for the latter than for the former group of bacilli.

COMPARATIVE READINGS.

In comparing the agglutinabilities of two emulsions which were being tested in parallel with each other, comparative readings by Madsen's method were always taken. A tube showing standard agglutination or thereabouts was chosen from one series and matched in the other, and the process was repeated once or more times with tubes showing rather more or rather less than standard agglutination. In calculating the results of the comparison, the average of the readings was taken. Table IX on p. 492 of this paper is the record of two typical experiments, and it shows the method of recording and calculating results used in this Laboratory.

The experiments recorded in this paper have been selected from a large material collected during nearly three years of experimental and routine work. It has only been possible to present a few of the most illustrative records, and in most cases the actual readings and the process of calculation have been omitted.

EXPERIMENTS WITH FORMALIZED BROTH CULTURES OF *B. DYSENTERIAE*.

Has the addition of 0.1 % formalin any immediate effect upon the degree of agglutinability of a broth-emulsion of *B. dysenteriae*?

The following experiments show that a practically immediate loss of sensitiveness may be caused by the addition of 0.1 % formalin.

Experiment I.

A culture of *B. dys.* Flexner in a litre of broth after 24 hours in the incubator was put in the cold chamber, and specimens were taken from

it at intervals. To each of these specimens 0.1 % of formalin was added. The opacity of the first specimen was determined in comparison with an old suspension of suitable thickness, and this sample and all subsequent samples were diluted to the right opacity with sterile formalized saline¹ and then put back at once in the cold chamber. The first specimen (A) was taken after the culture had been in the cold chamber for four hours.

The second (B) was taken fourteen hours later; the third (C) four hours after the second. The fourth (D) four hours after the third; the fifth (E) one hour after the fourth. Finally the remainder of the culture (F) was diluted with saline to the same opacity, and a parallel agglutination test with all specimens was put up just 24 hours after the taking of the first sample.

Thus, when the test was performed, (F) was unformalized, (E) had been formalized for two hours, (D) for three hours, (C) for seven hours, (B) for eleven hours, and (A) for twenty-four hours. All were tested with the same serum, and the figures given express the relative quantities of serum required to agglutinate the different emulsions to the same point (standard agglutination), the quantity of serum required by the unformalized culture being taken as unity. Each figure is calculated as the average of four comparative readings, two of which were taken after three hours in the water-bath, and two after a further 18 to 20 hours at room temperature.

TABLE I.

	Samples of Flexner culture and lengths of time since formalin (0.1%) was added to them					
	F	E	D	C	B	A
	No formalin	2 hours	3 hours	7 hours	11 hours	24 hours
Relative quantities of Flexner serum required to give standard agglu- tination	1.00	1.35	1.41	1.42	1.38	1.45

The table shows that the formalized samples A to E all require more serum to agglutinate them than does the unformalized culture F. Their sensitivity, then, has been lowered by the addition of formalin by about 40 % of its original (F) value. Within the first two hours there is an increase of 35 % in the quantity of serum required to cause standard agglutination, and in the next hour the increase mounts up to 41 %. After this time, the figures give us no certain evidence of further change.

If, as might be suggested, the apparent loss of sensitiveness of the formalized specimens were in fact due to a real increase of sensitiveness

¹ In this paper "saline" means 0.85 % NaCl solution in distilled water. "Formalized saline" means the same solution, to which 0.1 % commercial formalin has been added.

of the living culture during its 24 hours in the ice-chamber, then we should of necessity see a definite gradation of the figures; the most recently formalized being not far removed in sensitiveness from the untreated culture. This is, however, not the case.

Experiment II.

A similar experiment with a culture of *B. dys.* Shiga is given in Table II.

TABLE II.

					Samples of Shiga culture and lengths of time since formalin (0.1%) was added to them				
					E	D	C	B	A
					No formalin	1 hour	2 hours	17 hours	22 hours
Relative quantities of Shiga serum required to give standard agglutination					1.00	1.04	1.03	1.11	1.12

Here we see a loss of sensitiveness similar to that in Table I, but not so great. Moreover the loss appears to have been more gradual in this case, since there is a pronounced difference between the samples that had been formalized for one to two hours and those that had been formalized for 17 and 22 hours respectively. A number of similar experiments, either with *B. dysenteriae* Shiga or with *B. dysenteriae* Flexner, were performed. In the case of *B. dysenteriae* Shiga in one experiment the addition of 0.1 % formalin did not give rise within eight hours to any measurable loss of sensitiveness. But in the experiments with *B. dysenteriae* Flexner, a pronounced loss always occurred during that period amounting in one case to over 50 % of its original value.

What is the further progress and duration of the change caused by the addition of 0.1 % formalin?

The experiments described below demonstrate the fact that the change is variable both in degree and in duration, that it comes to an end within a period of not more than two months from the addition of the formalin; and that after that point sensitiveness of the culture remains constant for an indefinite period (at least 9 to 12 months).

METHOD.

In investigating the question of change of sensitiveness of an agglutinable emulsion of bacilli over a period of time it is necessary to have some fixed standard or criterion by which the results of the successive tests may be judged and compared with one another. For this purpose

the ideal criterion is a standard agglutinable emulsion of known and unchanging sensitiveness, with which the new emulsion may be compared at intervals.

But since it is the whole object of this research to establish the possibility of preparing such a standard, we are driven for the time being to other devices.

I. A specific agglutinating serum may be assumed to keep a steady titre over a period of three months or so if it be stored in the ice-chamber and not used too fresh.

Common experience with immune sera teaches that they nearly always undergo a progressive loss of strength. Many series of experiments with freshly made agglutinating sera done in the course of this work have shown that the loss of power is the more rapid the fresher the serum is. After two months or so the rate of degeneration is usually so low as to be inappreciable in a further period of three or four months. Many specimens of serum, in fact, seem to arrive at such a state of stability that for twelve months or more no diminution of their agglutinating power can be detected. If a serum gives with a particular agglutinable emulsion the same readings at the beginning and at the end of a considerable period of time, the simplest conclusion we can draw is that neither the serum nor the emulsion has altered during the time. For instance, an emulsion of *B. dysenteriae* Y (more than four months old) was tested with a serum specific for that organism on Dec. 30, 1916. The quantity of serum required to cause standard agglutination was found to be 0.00177 c.c. Both serum and emulsion were kept in the cold chamber for six months, and on June 28, 1917, the test was repeated. On this occasion the quantity of serum required was 0.00173 c.c.

Since almost exactly the same quantity of serum agglutinated the same volume of emulsion to the same degree, we must conclude either that both serum and emulsion have remained constant, or that while the serum has been losing in strength, the emulsion has gained in sensitiveness to an exactly corresponding degree.

A third theoretical possibility, that the serum may have increased in strength, while the culture lost sensitiveness, may be ruled out on our general knowledge that sera do not increase in agglutinating power.

The second alternative is only partially dismissed by the improbability that exactly equal and opposite changes should have occurred in the two fluids. It is, however, sufficiently discounted by the fact that two years of experimental work on this subject have failed to produce any evidence that formalized suspensions of dysentery bacilli ever undergo

an increase of sensitiveness. So the balance of evidence is very strongly in favour of the first explanation, viz., that neither the serum nor the culture has undergone any appreciable change of its properties during the period of the experiment. The experiment just mentioned sufficiently demonstrates the complete stability of the materials (serum and emulsion) employed, and raises a presumption that other sera and emulsions, made and kept under similar conditions, will show a similar stability. Other instances of the same phenomenon will be found below.

For the purpose of testing the stability of agglutinable emulsions of dysentery bacilli, an old stock agglutinating serum may be used as a provisional standard and its stability over a period of some months may be provisionally assumed.

II. There is a second method of testing an agglutinable emulsion for changes in its agglutinability.

An old emulsion, whose stability has been provisionally demonstrated in the manner just described, may be taken as standard, i.e., may be assumed to be stable during the period of the experiment. The new emulsion which is being investigated is tested at intervals in parallel with the old emulsion. If the new emulsion be in process of losing sensitiveness, an increase will be seen in the relative amount of serum required to agglutinate it to the same degree as the old emulsion. In such an experiment it is not necessary to use the same specimen of serum in all the consecutive tests; any specimen of specific serum will do, because it is only the relation of the quantities required by the two cultures that interests us.

If the relative quantities of serum remain unchanged, we must conclude that the new emulsion has undergone no alteration.

III. The ideal method consists of a combination of the two methods just described.

EXPERIMENTS TO DETERMINE THE COURSE OF THE LOSS OF SENSITIVITY CAUSED BY THE ADDITION OF FORMALIN.

(1) A 24 hours bouillon culture of *B. dysenteriae* Shiga was diluted with saline to standard opacity, and its exact relative degree of sensitiveness to Shiga agglutinating serum was determined in comparison with that of a stock formalized emulsion (Sh. 1). Formalin (0.1 %) was then added, and the culture was put into the cold store.

Samples were taken out at intervals and tested, as before, against the stock emulsion, to determine and measure any change that might be occurring in the agglutinability of the new culture.

Table III and Chart I give the results of these tests. In the right-hand column there are recorded a number of tests of the stock Shiga emulsion with a particular specimen of Shiga serum. It is seen that the quantity of this serum required to give standard agglutination has not changed during the period of the experiment, and therefore it may be assumed that the agglutinability of Sh. 1 (stock emulsion) has not altered.

TABLE III (Chart I).

Date	Relative quantities of serum required to give standard agglutination		Quantities of a single specimen required to give standard agglutination with the stock culture
	Stock culture	New culture	
11. xii. 15			
Before adding formalin	100	57.8	0.00143 c.c.
11. xii. 15			
After adding formalin	100	63.4	—
20. xii. 15	100	99.0	—
4. i. 16	100	115.0	0.00146 „
25. i. 16	100	114.5	—
3. iv. 16	—	—	0.00141 „
25. v. 16	—	—	0.00143 „

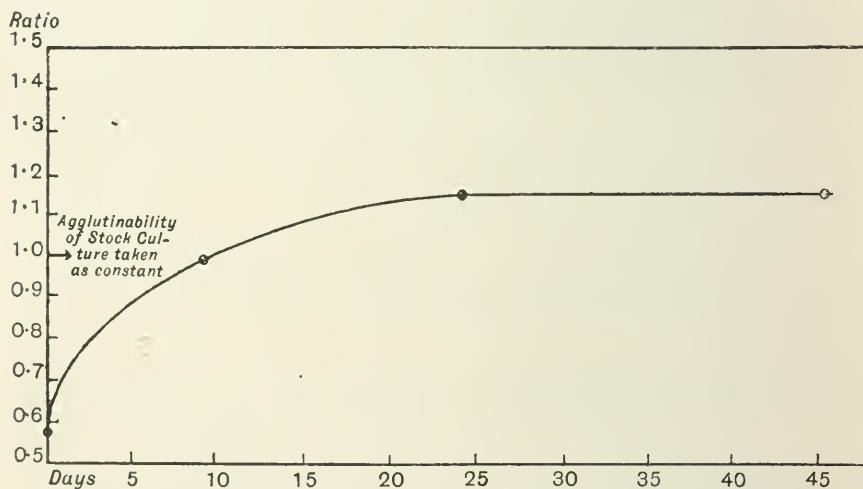


CHART I. Curve showing the increase of the ratio of the quantity of serum required to give standard agglutination with a new formalized broth culture of *B. dysenteriae* Shiga to the quantity required by an old stock culture of constant agglutinability.

Ordinates. Ratios of the quantities of serum required to agglutinate the two cultures to the same degree.

Abscissae. Time, in days.

Note. The rise of this ratio signifies a fall of the agglutinability of the new culture as compared with the old.

This experiment demonstrates that the particular fresh formalized Shiga culture in question needed during the first 24 days of its existence a progressively increasing quantity of serum to agglutinate it to the same degree as the stable stock culture. In other words it underwent during that period a progressive loss of sensitiveness. Between the 24th and the 45th days no further loss was shown to have taken place. The third observation in the table (20. xii. 15) included also a test of the remainder of the original unformalized and still living fresh Shiga culture, which had been kept on ice. In contrast to the formalized specimen it showed, if any, only a slight alteration of sensitiveness, and that in the opposite direction, viz., the relative quantity of serum required to agglutinate it to the same degree as the stock culture had altered from 57.8 to 56.5. Increases of apparent sensitiveness in unformalized living cultures are always liable to occur owing to lysis of the bacteria and consequent thinning of the emulsion. This may or may not be over-compensated by the continued growth of the live bacilli, according to the degree of cold and other conditions under which the emulsion is stored.

(2) A fresh emulsion of *B. dysenteriae* Flexner was made and treated exactly in the way described in the foregoing experiment. Table IV and Chart II show the changes undergone by the freshly formalized emulsion as compared with old stock Flexner culture. A few tests of the unformalized living culture are included in a separate column of the table.

In this table and Chart II new formalized Flexner culture shows a decrease of sensitiveness, at first rapid and then gradually becoming slower, in the first 22 days of its existence, after which period it shows no further alteration of agglutinability during the subsequent 26 days.

The right-hand column of the table presents good evidence of the stability of the stock culture which was used as the basis of comparison.

The loss of sensitiveness of the unformalized, living culture may well have been due to a slow continued growth of the bacillus, and the consequent increase of the quantity of bacterial substance in the emulsion.

The foregoing experiments suffice to prove (1) that formalized cultures of *B. dysenteriae* may undergo an early loss of sensitiveness, and (2) that the change may run its complete course in some three weeks, after which period the agglutinability undergoes no further change. For how long this stability is maintained a later section of this paper will show.

It must be observed at this point that formalin does not invariably cause a decline in sensitiveness of dysentery cultures. Sometimes a culture is found to be stable from first to last; and this is more often

TABLE IV (Chart II).

Date	Relative quantities of serum required to give standard agglutination with cultures of <i>B. dysenteriae</i> (Flexner)			Quantities of a single specimen of Flexner serum required to give standard agglutination with the stock culture
	Stock culture	Formalized new culture	Unformalized new culture	
19. xi. 15	100	84.0	72.5	0.056 c.c.
29. xi. 15	100	182.0	96	—
30. xi. 15	100	172	102	—
11. xii. 15	100	311	—	0.054 „
6. i. 16	100	310	—	0.054 „

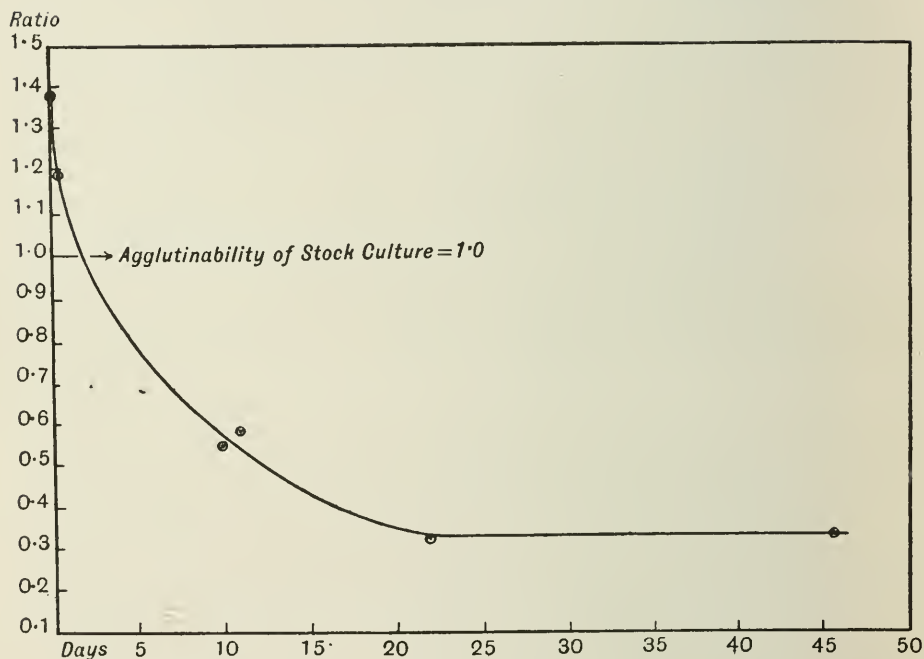


CHART II. Curve showing the fall of the ratio of agglutinability of a new formalized broth culture of *B. dysenteriae* Flexner to that of an old stock culture of constant agglutinability.

Ordinates. Ratios of agglutinability of new culture to that of old culture of the same bacillus.

Abscissae. Time, in days.

Note. The ratios of agglutinability are the reciprocals of the ratios of the quantities of serum required. A curve plotted with the latter values would have an upward direction similar to Chart I.

the case with *B. dysenteriae* Shiga than with the mannite-fermenting group. Table VIII shows a series of tests of a Flexner culture which yielded no certain evidence of any loss of agglutinability from the moment after the addition of formalin to the last test nearly five months later. The mere addition of formalin to the fluid almost invariably lowers the agglutinability by some few per cent., but the progressive change seen in the experiments given above does not always ensue. The explanation may lie in the degree of cold in which the culture is stored during the early period while the formalin is taking effect. The cold store made use of for the purpose of this investigation was kept at an average temperature of 40° F., and at times was a little warmer, at other times colder than that. It is possible that a constant temperature only just above the freezing point of water as recommended in the Directions for the Preparation of Standard Cultures would appreciably reduce the frequency and degree of these changes.

DURATION OF THE LOSS OF SENSITIVENESS CAUSED BY FORMALIN.

Different batches of cultures show considerable variations in their period of stability. We have seen that in some cases no loss of sensitiveness at all occurs, while in others the culture takes a month or more to reach a constant level of agglutinability. No case has yet occurred within my experience of a continuation of loss of sensitiveness beyond the end of the second month. Nevertheless I have adopted the routine plan of considering every batch of culture as "unripe" until it is three months old.

THE STABILITY OF "RIPE" CULTURES.

When once the curve of sensitiveness has reached a constant level, it remains at that level for an indeterminate period.

Tables V-VIII give instances of a number of fresh dysentery cultures whose sensitiveness was determined at intervals in comparison with an old "ripe" culture. Table V gives a series of determinations of a fresh Flexner culture. The figures in the two middle columns may be read on this plan: If 100 parts of a serum are needed to give standard agglutination with the standard culture, 82 parts of the same serum are required to give the same degree of agglutination with the new culture, i.e., the new culture, at the first determination, was more sensitive than the standard culture, in the proportion of $100/82 = 1.22 : 1$.

TABLE V.

Periodical tests of a new B. dysenteriae Flexner culture against standard culture of the same organism. Both cultures made with the same strain of B. dysenteriae Flexner.

Age, in weeks of culture under standardization	Relative quantities of serum required to give standard agglutination with		Average figure for monthly periods
	Standard culture	Culture under standardization	
4	100	82	82
8	100	95	—
8—9	100	104	100
8—9	100	97	—
10	100	103	—
14—15	100	114	—
15—16	100	101	106
15—16	100	102	—
21—22	100	104	104
49—50	100	116	—
49—50	100	105	103
49—50	100	94	—
49—50	100	101	—

The standard culture was more than twelve weeks old at the date of the first test.

Considering next the average figures for monthly periods, we find that this ratio of sensitiveness has become 1.00 in the 8th–10th weeks—i.e., the new culture has lost sensitiveness (assuming that the standard culture has not gained). The three subsequent ratios are 0.94, 0.96 and 0.97, and it will be noted that the average for the 49–50 weeks is only 3 % greater than that for the 8th–10th weeks.

We cannot therefore conclude for certain that any loss of sensitiveness has occurred after the eighth week. From that point onwards, until the 50th week, the relative agglutinabilities of the two cultures remained unaltered.

Table VI presents a similar series of experiments with two cultures of *B. dysenteriae* Y. In this case no alteration of agglutinability was found in the new culture between the 4th and the 31st weeks. No further determinations were done, as the stock standard culture had all been used up.

Table VII contains the figures obtained by a series of tests of two Shiga cultures. Culture (A) was already six months old at the time of the first test, and culture (B) was slightly more than three months old. The period of the experiment is some six months. Since the variations in the relative observed values do not exceed 6 % on either side of the

mean value (53) it is to be concluded that no significant alteration has taken place.

TABLE VI.

Periodical tests of a new "Y" culture against a standard culture of the same organism. Both cultures made with the same strain of "Y."

Age, in weeks of culture under standardization	Relative quantities of serum required to give standard agglutination with		Average figure for monthly periods
	Standard culture	Culture under standardization	
4—5	100	69	—
4—5	100	76	73
5—6	100	73	—
17—18	100	72	72
30—31	100	76	—
30—31	100	78	—
30—31	100	76	73
31	100	60	—

The standard culture was more than six months old at the time of the first test.

TABLE VII.

Determinations of the relative sensitiveness of two Shiga emulsions.

Date	Relative quantities of serum required to give standard agglutination with		No. of standard serum-units re- quired to give standard agglu- tination with culture (A)
	Shiga culture (A)	Shiga culture (B)	
29. v. 17	100	56	1.9
9. viii. 17	100	52	1.7
31. x. 17	100	50	1.7
11. i. 18	100	54	1.8

Both cultures were made with the same strain of *B. dysenteriae* (Shiga) and both were more than three months old at the time of the first test.

The fourth column gives the number of standard serum-units which were found on the various occasions to give standard agglutination with culture (A). Different specimens of Shiga serum were used for the different tests, and the strength in units of each serum was controlled by testing them against a third and older batch of standard culture. The closeness of the figures obtained thus, provides an additional proof of the stability of the culture (A), which was used as standard.

Table VIII is in most respects quite similar to the foregoing tables. One point however must be mentioned. Two different standard cultures were used in this series of tests. Standard culture (X) was employed

for the first two, and standard culture (Y) for the remainder (X having run out). But the two standard cultures had been thoroughly and repeatedly tested against each other, and a reduction-factor obtained

TABLE VIII.

Date	Relative quantities of serum required to give standard agglutination with		Averages of ratios
	Standard Flexner culture	New Flexner culture	
6. iv. 16	100	172	—
14. iv. 16	100	161	169·3
18. iv. 16	100	175	—
28. viii. 16	100	170	169·5
29. viii. 16	100	169	—

Note. The new culture was made on 5. iv. 16, and formalin was added on 6. iv. 16. Both cultures were made with the same strain of *B. dysenteriae* Flexner.

TABLE IX.

Two tests in parallel of two standard "Y" cultures. Different specimens of serum used on the two occasions.

Date	Readings		Quantity of serum		Ratio	
	Y 3	Y 4	Y 3	Y 4	Y 3	Y 4
7. iv. 17						
4½ hours	6— <u>7</u>	4 S	3·2	5·0	100	156
	7 S—	5	3·0	4·0	100	133
	8	6 tr +	2·5	3·5	100	140
	9	7 tr —	2·0	3·0	100	150
24 hours	10 tr —	9	1·5	2·0	100	133
					5) 500	5) 712
					100	142
Date	Y 3	Y 4	Y 3	Y 4	Y 3	Y 4
4. vii. 17						
4½ hours	4 S +	1— <u>2</u>	5·0	8·2	100	164
	5 S —	<u>3</u> —4	4·0	5·9	100	148
	5— <u>6</u>	4 tr	3·6	5·0	100	139
	7 tr +	5	3·0	4·0	100	133
24 hours	8	6 tr	2·5	3·5	100	140
					5) 500	5) 724
					100	145

Note. The first reading of the first test means: "Tube 4 of series Y 4 shows more agglutination than tube 6 and less than tube 7 in the other series. It is judged to be rather nearer tube 7." The quantity of serum to which this corresponds is less than 3·5 and more than 3·0; nearer the latter than the former, i.e., 3·2. So with the other interpolated readings. One to four marks under one of a pair of figures signify that the reading was judged to be slightly or very much nearer to the underlined tube.

with which it was easy to translate the results with culture (X) into terms of culture (Y). It is in fact this operation that is the main purpose of the standardization of agglutinable cultures.

As additional illustrations of the stability of cultures over long periods the following may be mentioned.

A specimen of *B. dysenteriae* Y agglutinating serum, tested with a standard Y culture, was found to contain 538 standard units per c.c. Just over six months later the test was exactly repeated, and the serum showed an agglutinin constant of 545 standard units.

Evidently if the culture had undergone any alteration of sensitiveness in either direction, the number of serum units calculated could not have been practically identical on the two occasions.

An exactly similar experiment with a different Y standard culture (the same strain of Y) and a different sample of serum gave the following results.

In the first test it was found that 0.00085 c.c. of serum gave standard agglutination with the culture. Three months later the quantity was found to be 0.00082 c.c.; and again three months later 0.00087 c.c. Experimental error is quite sufficient to account for the differences between these figures, none of which varies so much as 4 % from the mean of the three.

Again, on 30. x. 16, 0.0020 c.c. of a Shiga serum was found to give standard agglutination with a Shiga culture. Nearly ten months later, on 10. viii. 17, the test was repeated, and 0.0021 c.c. of the serum was found to give standard agglutination with the same culture.

It is hardly necessary to multiply these illustrations, which are drawn from a large material. Those given are sufficient to show that formalized broth cultures of the various dysentery bacilli, after a period of "ripening," during which their agglutinability is diminished in varying degree, remain stable for at least ten months. There is, moreover, every reason to believe that the life of an emulsion is much longer than this, and it may in fact extend, as in the case of the best typhoid or paratyphoid cultures, to a number of years.

PRACTICAL SIGNIFICANCE OF THE ACTION OF FORMALIN.

The foregoing experiments prove that (1) stable standard agglutinable cultures can be made from the various dysentery bacilli. (2) such cultures cannot be standardized for about two months after they are made. For during this period a progressive loss of agglutinability may occur.

Where agglutinable cultures are made on a large scale and stored until required, these considerations constitute no obstacle to the manufacture of standard cultures by the formalin method, for it is a simple matter to lay in stocks which will not come into use for three months or more.

Moreover if any bacteriologist should prefer to make his own formalized broth cultures and use them as soon as made the course is always open to him of determining their agglutinability at intervals of one or two weeks against a ripe stock standard culture during the period of change. Unless this is done the results of tests performed at intervals of more than a few days cannot be regarded as quantitatively comparable.

THE CONSTANT (K) OF AGGLUTINABILITY AND ITS SIGNIFICANCE.

Having determined the conditions under which formalized dysentery cultures reach and maintain a lasting level of agglutinability, we are in a position to apply to these cultures the whole system of standardization which was instituted by Prof. Dreyer for the typhoid and paratyphoid bacilli.

The principles may be stated thus.

(1) Every emulsion of bacilli has its own specific agglutinability. Differences of hundreds per cent. are possible in emulsions made with the same strain of bacillus.

(2) The observed end-point of an agglutinin reaction is conditioned by this factor, and therefore if the factor remains undetermined, the observed end-point is no real measure of the strength of the serum but merely a record of what happens with that particular emulsion.

(3) Emulsions made by the broth-formalin process have a long life of stable agglutinability. This makes it possible to choose an emulsion as an arbitrary standard, and to determine the relative agglutinability of any number of similar emulsions as compared with the standard. Each emulsion is thus provided with a numerical factor or constant of agglutinability, i.e., a figure which expresses how much more or how much less sensitive it is than the standard.

If a uniform technique be adopted in which a definite volume of serum in graded dilutions is made to act upon a definite volume of standardized culture at a definite temperature for a specified time, then the results of any number of tests performed with different standardized emulsions may be rendered quantitatively comparable by dividing the

dilution-figure of the end-point of each reaction by the agglutinability-constant of the emulsion employed.

The figure so obtained is most conveniently expressed as "agglutinin units."

For instance, a Shiga serum, tested with two different Shiga emulsions, is found to give standard agglutination with the one at a dilution of 1/1000, with the other at 1/1800. The former emulsion is chosen as arbitrary standard, and its K of agglutinability fixed as unity. The number of agglutinin units contained in the serum is obtained by dividing the denominator of the dilution-fraction, i.e., 1000, by the agglutinability-constant, i.e., 1.0.

Thus the serum is said to contain 1000 standard agglutinin units per c.c. The second emulsion gave standard agglutination at 1/1800, which means that it is 1.8 times more agglutinable than emulsion No. 1. Since 1800 divided by 1.8 gives 1000, it is clear that 1.8 must be the agglutinability-constant of the second emulsion.

So, by the use of these constants, we obtain the same number of agglutinin units for the serum, whereas, by merely expressing our results as dilutions, we had arrived at quite different figures.

In practice the K of the original arbitrary standard culture is not fixed as 1.0, but at some higher figure. For with a K of 1.0, the unit is small, and the number of units in any given serum is rendered relatively large. Many normal and non-specific sera would thus be made to contain a considerable number of agglutinin units, whereas it is desirable that such sera should contain few or no units. By fixing the original K at 2.5, as was done by Prof. Dreyer in the case of typhoid and paratyphoid cultures, or at 3.0 (*B. dysenteriae* Flexner and Y) or at 2.0 (*B. dysenteriae* Shiga) the respective agglutinin units are made larger than they would have been if a K of 1.0 had been chosen, and thus the number of units contained by all sera are proportionally reduced.

Normal and non-specific human and rabbit sera are thus made to have a low unit-content, which is convenient when determining a diagnostic level for the specific rise of agglutinins in active infection, whether it be enteric fever or bacillary dysentery.

THE STANDARD AGGLUTININ UNIT.

Prof. Dreyer's standard agglutinin unit for the typhoid-paratyphoid group of bacilli is defined as "that quantity of an agglutinating serum which, when made up to 1.0 c.c. volume with normal saline solution, causes standard agglutination on being mixed with 1.5 c.c. of a particular

standard agglutinable culture and maintained at 55° C. for two hours in a water bath, followed by 15 minutes at room-temperature.

It is to be noted that the method of measurement by drops is so arranged that the results can be read off directly as though the measurements had been performed in cubic centimetres. For ten drops of diluted serum always act upon 15 drops of culture, and the result is actually the same as that obtained with 1.0 c.c. of diluted serum and 1.5 c.c. of culture, provided that the size and shape of the tubes used in the two cases are proportional to the different volumes of fluid. For, as Ainley-Walker (1916) has shown, the differences in drop-sizes compensate for one another in this technique, so that the error from this cause is reduced to negligible proportions. It follows from the definition of standard agglutinin unit that the particular standard agglutinable culture referred to must be given a K of 2.5. For 1 c.c. of a serum which contained one unit per c.c. gave standard agglutination when mixed with 1.5 c.c. of the culture; i.e., standard agglutination occurred at a serum-dilution of 1/2.5. Now since the number of units per cc. in the serum

$$= \frac{\text{Dilution in which standard agglutination occurs}}{K \text{ of culture}},$$

we have

$$1 = \frac{2.5}{K},$$

or

$$K = 2.5.$$

Adopting for dysentery agglutination the definition of standard agglutinin unit given above, with the single alteration of two hours to four and a half hours, we must also give to our original basis emulsion of each strain the K of 2.5. But since, for the purely practical purposes already mentioned, we may wish to have higher or lower K 's on an average for one or other strain of bacillus, we are at liberty to give our first real emulsion some figure higher or lower than 2.5, and make the theoretical assumption that the figure has been arrived at by standardizing this emulsion against a (fictitious) original standard culture whose K was 2.5. Such a procedure was adopted in the case of the various dysentery bacilli. A large number of normal human sera were tested by Prof. Dreyer and the writer (the results of which will appear in a separate communication) in order to determine the normal human limits of agglutination with standard cultures.

Since normal sera were found to contain more agglutinin for *B. dysenteriae* Flexner than for *B. dysenteriae* Shiga, the K for the former was placed high (3.0) and that for the latter low (2.0), which figures

rendered the number of units present in normal sera for the two organisms approximately equal. This had the advantage of enabling a single inclusive figure to be given for the diagnostic level of agglutinin units for the two types of bacilli in clinical serum tests.

A suitable K for the first emulsion of a bacillary strain having been determined, all subsequent emulsions are standardized by repeated testing in parallel with the first.

In the routine standardization of dysentery cultures in the Standards Laboratory a new emulsion is always tested at least six times with the full series of tubes against two or more previous standard emulsions. A number of these tests, never less than four, are performed shortly before the emulsion is to come into use, at which time it is at least three months old. Thus the K is calculated as the average of anything from 24 single readings upwards; usually many more than this. By this means experimental error and errors due to sampling, etc., are overcome, and a correct K can be determined. One of the most troublesome sources of error under present conditions is found in the variable alkalinity of the glass of different batches of bottles, which sometimes causes an appreciable variation of agglutinability of different samples of the same emulsion.

In spite of the greatest care in the preparation of bottles, including prolonged treatment with acid, it has not yet been found possible entirely to eliminate this trouble, which seems to be one of those inherent in war-conditions. In the calculation of the K of an emulsion it can only be overcome by testing a number of different bottles of culture and striking an average. For the purpose of the clinical test the error is of no great significance, since it rarely exceeds a 10 % difference between individual bottles.

EXPERIMENTS WITH HEAT AND WITH ANTISEPTICS OTHER THAN FORMALIN.

Although the use of heat and of certain antiseptic substances other than formalin has been demonstrated to be greatly inferior to formalin in the preparation of agglutinable cultures of *B. typhosus* (Aaser 1905, Dreyer 1906), it does not follow that the same is the case with cultures of *B. dysenteriae*. For the latter, as we have seen, may undergo a diminution of agglutinability under the influence of formalin, while the former do not.

In order to discover whether there might be a better method of preparing stable emulsions than the formalin procedure, a series of experiments were carried out with emulsions prepared in the following ways.

Broth cultures were always used, since saline emulsions from agar have been demonstrated by Dreyer in the case of *B. typhosus* to be greatly inferior, and since they failed to yield a satisfactory permanent "diagnosticum" in the hands of Schiemann.

METHODS OF PREPARATION EMPLOYED.

- (1) Heating to 58° C. for 1 hour to 1½ hours.
- (2) " " " " and adding thymol.
- (3) " " " " " chloroform.
- (4) " " " " " 0.1 % formalin.
- (5) Simple addition of thymol to unheated culture.
- (6) " " " chloroform.
- (7) " " " phenol 0.5 %.
- (8) " " " " 0.3 %.

The thymol and chloroform were added in such quantities as to saturate the solution, leaving a visible excess in the bottle.

The various emulsions were diluted to the right opacity with salt solution, and were immediately tested for changes in agglutinability against a control untreated emulsion made from the same culture and diluted to the same opacity. One or more "ripe" formalized cultures of the same bacillus were included in the test, as a basis from which the agglutinabilities of the various cultures could be calculated. The cultures were (with the exception of No. 8) kept in the cold store when not being tested. The whole test was repeated at intervals, in order to ascertain what changes in agglutinability were in progress.

SUMMARY OF RESULTS.

- (1) *Heating to 58° C. for 1 hour or 1½ hours.*

A 24 hours culture of *B. dysenteriae* Flexner was treated in this way. A test immediately after heating showed the heated culture to have suffered a reduction in agglutinability amounting to slightly over 30 %. Repeated tests during the subsequent nine weeks showed no further alteration.

There was noticeable, however, an alteration in the appearance of the emulsion at the end of this period, a bluish translucency which suggested that some autolysis or disintegration of the bacilli had occurred. As we shall see, this change is constant in emulsions heated to this degree and not subjected to fixation with formalin.

A second Flexner culture was treated in the same way. The test immediately after heating showed that the heated culture had lost none

of its agglutinability; and subsequent tests during the next five weeks showed an average loss of less than 5 %. But by this time clearing of the emulsion was becoming evident, as in the case given above.

Had further tests been possible over a longer period of time, there would doubtless have been a slow apparent increase of agglutinability, due to the disintegration of the bacilli, as we shall see in the next experiments.

(2) *Heating to 58° C. and adding thymol.*

A Flexner culture prepared in this manner was found to have suffered an immediate loss of sensitiveness amounting to 45 %.

No further loss was demonstrated in a series of tests during the following four weeks. Later tests during the second month showed a tendency towards an increase of sensitiveness, and in a final test performed four months after the preparation of the culture, a definite increase was established, the loss of sensitiveness being now reduced from 45 % to 13 %. Coincident with this change there was a very noticeable clearing and translucency of the emulsion.

It is worth while mentioning that the addition of thymol to a fluid has a profound effect on the surface tension, with the result that the size of the drop is greatly reduced. Drop-measurements of thymolized fluids are therefore inadmissible, unless corrective calculations are made.

(3) *Heating to 58° C. for 1 to 1½ hours and adding chloroform.*

A 24 hours broth culture of *B. dysenteriae* Flexner was subjected to this treatment. The result was in every respect similar to that of the last-mentioned experiment, viz., an immediate considerable loss of agglutinability (40 %) followed by a short period of apparent stability, and later by a rise accompanied by a progressive diminution of the opacity of the culture.

Conclusion. Heating to 58° C. for 1 to 1½ hours, with or without the addition of thymol or chloroform, is insufficient to fix the bacilli, and so to prevent their slow disintegration. Permanent and stable emulsions cannot be made in this way.

(4) *Heating to 58° C. for 1 to 1½ hours and adding 0·1 % formalin.*

The Flexner culture used in this experiment showed a loss of 18 % directly after the treatment. Another specimen of the culture, treated with 0·1 % formalin only, gave evidence of very little loss (2·2 %). The observations were only continued for six weeks, at the end of which time

no further change of agglutinability had been detected, and the appearance and opacity of the culture were unaltered.

Conclusion. The combined heat and formalin method is inferior to formalin alone.

(5) and (6) *Simple addition of thymol or chloroform to the unheated culture.*

A progressive loss of sensitiveness was found in the specimens of Flexner culture treated in this way. But it was probably due to continued growth of the bacillus, for sub-cultures taken two days after the antiseptics were added in both cases showed the presence of live bacilli, and even 17 days later the chloroformed sample gave a positive subculture. At the end of eight weeks very pronounced clearing of the culture betrayed the fact that disintegration of the bacilli had been taking place for some while, and the tests were, on this account discontinued. These series of tests showed considerable fluctuations, which were difficult to interpret.

Conclusion. Owing to slow or incomplete sterilization and absence of fixation of the bacilli this mode of preparation proved to be totally unsatisfactory.

(7) *Simple addition of 0.5 % phenol combined with cold-storage.*

To a living 24 hours broth culture of *B. dysenteriae* Flexner 0.5 % phenol was added, and the culture was at once tested for its agglutinability against untreated sample which had been kept for a control.

The test showed a loss of sensitiveness amounting to 50 % of its original value (i.e., double the quantity of serum was required to give standard agglutination).

This culture was not tested further.

Another culture similarly treated, but whose initial loss was, by an oversight, not determined, showed a period of stability during four weeks of periodical tests. No examination was made until this culture had stood a further five months in the cold store. The tests then carried out demonstrated an increase of agglutinability amounting to 22 % of its previous value. There was no noticeable decrease of opacity. These facts suggest that 0.5 % phenol causes a partial, but incomplete, fixation of the bacilli, which disintegrate very slowly. Any clearing of the fluid due to this disintegration may be compensated by a continuous slow precipitation of dissolved albuminous bodies.

(8) *The addition of 0.3 % phenol (culture stored in the cold for 24 hours thenceforward at room temperature).*

A Shiga culture, treated in this way, underwent no immediate loss of

sensitiveness. Nor were the subsequent changes of any great magnitude. They exceeded by little the experimental variations.

The figures however suggest a decrease of sensitiveness visible at the end of five or six weeks, and followed by a secondary increase. At the end of the experiment clearing of the culture had begun to be noticeable. So that, even if the agglutinability had been unequivocally steady up to that time, the culture could not be considered as satisfactory.

TABLE X.

Date	Quantities of serum required to give standard agglutination with			Relative serum quantities giving standard agglutination with	
	Standard Shiga culture	New Shiga culture un- treated	New Shiga culture with 0·3% phenol	Standard Shiga culture	New Shiga culture with 0·3% phenol
15. xii. 17	·0022 c.c.	·0017 c.c.	·0017 c.c.	100	77
17. xii. 17	·0024 „	—	·0017 „	100	71
20. xii. 17	·0020 „	—	·0014 „	100	70
17. i. 18	·0019 „	—	·0014 „	100	74
18. i. 18	·0023 „	—	·0018 „	100	78
28. i. 18	·0023 „	—	·0019 „	100	83
21. ii. 18	·0023 „	—	·0017 „	100	74
22. iii. 18	·0022 „	—	·0016 „	100	73
22. iii. 18	·0022 „	—	·0016 „	100	73

Conclusion. Thus phenol by itself (0·5 % or 0·8 %) is insufficient for the preparation of standard agglutinable cultures whose agglutinability has to be guaranteed as constant over comparatively long periods of time. Unlike formalin, it fails to fix the bacilli and so to prevent their slow disintegration and solution.

SUMMARY.

(1) Killed agglutinable broth cultures of the various dysentery bacilli may be prepared by the formalin and cold storage process and may be standardized by the method elaborated by Prof. Dreyer for the typhoid and paratyphoid bacilli.

(2) Since formalin (0·1 %) usually causes a gradual loss of sensitiveness to agglutination during the early life of the emulsion, a certain period (two months) must be allowed to elapse before the emulsion can be used as standard.

(3) After such a period the sensitiveness of the emulsions remains unaltered for at least ten months, and probably much longer.

(4) By none of the other methods of preparation investigated could satisfactory standard agglutinable cultures be made.

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